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STRUCTURE AND GERMINATION OF SEPTORIA SPORES[1]

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INTRODUCTION

An intimate knowledge of the structure of spores of pathogenic fungi is of major importance to agricultural practice because of the relation of spore structure to method of infection and possibly to the effectiveness of control measures. Such knowledge has a definite bearing on the development of effective chemical methods of preventing or controlling certain plant diseases. Current methods commonly utilize spores of different fungi as test material in determining the probable value of new and improved chemicals as fungicides. The mode of action and the effectiveness of specific substances as fungicides depend in part upon the structure and the organization of the spores with which they come in contact.

Septoria apii-graveolentis Dorogin, the cause of late blight of celery (*Apium graveolens* L.), is widespread on cultivated and escaped celery in California. The general appearance of the disease on cultivated celery is somewhat different from that on escaped celery, but a study of the fungus on the two types of host failed to reveal any significant morphological differences. This study led to a further study of the spores of this species of *Septoria*, with emphasis on the detailed structure of the spores, the meaning and nature of septation, the meaning and nature of guttulae, the variation in the form of the spores, the structural changes due to growth and germination, and the meaning of maturity. The results of this study are reported herein. These observations have a bearing on the structure of *Septoria* spores in general, and this is briefly discussed.

MATERIAL AND METHODS

The spores of *Septoria apii-graveolentis* are about 30μ to 50μ long and 1μ to 2.5μ in diameter. The spores have a highly elaborated and detailed structure, which can be observed only at high magnifications. The details of the structure cannot be delineated in the bright field alone, and it is only by the use of the dark field under the most favorable circumstances that structure is revealed more completely. Observations made in the bright field alone are incomplete and sometimes erroneous. The spores as seen under the microscope are too small to photograph or to draw satisfactorily with the aid of the camera lucida. The drawings reproduced herein represent the spores as actually seen

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and were prepared with precise attention to details of size, form, and relative position of the spore constituents. Projection at high magnification in a darkened room was a substantial aid in the work.

Celery leaves and leafstalks bearing pycnidia were selected in the field from either escaped or cultivated celery. Plants that showed reduction in vigor due to age or changes due to or symptoms of other diseases were never used. The leaves and leafstalks were put in the refrigerator at a temperature of 10° C. In a few hours spore horns (cirri) exuded from the pycnidia. Fresh spore horns provided the most uniform source of spores; they were free of extraneous matter and remarkably uniform in size and general condition. Unless spores from within the pycnidium were desired for special reasons, the spores of the spore horns were used throughout the study. After sufficient observations had been made to determine when *Septoria* spores are mature, only mature spores were used.

THE SPORE HORN, OR CIRRUS

The spore horn exudes from the ostiole of the pycnidium under some pressure as a threadlike structure, more or less curled. The amount of curling of the spore horn can be assigned to no specific cause, but it is believed to be due to conditions within the pycnidium and to the rapidity with which drying takes place as the horn emerges. The cross section of the horn is generally circular, but it may vary and conform somewhat to the shape of the ostiole. The length of the horns is variable. Some are only 2 to 5 mm. long, especially on thin leaves. Horns exuding from pycnidia embedded in the surface tissue of leafstalks are frequently 1 to 1.5 cm. long and occasionally as much as 2 cm. long. The color when freshly exuded is a light tan. The spores in the spore horn are held by some mucilage that is not seen. When a horn segment is placed in water the release of the spores from each other is immediate and complete, and no trace of any matrix or cementing substance can be observed. In the dry state the spores lie in the horn with their long axis parallel to that of the horn. The more slender or pointed end of the spore is the point of attachment within the pycnidium, and the spores are always oriented in the horn so that the distal or less pointed end emerges first. Although the ends of the spores are not markedly different in shape, there is ordinarily no difficulty in distinguishing the distal from the proximal end.

In an immature pycnidium the spores are bent and more crooked, sometimes with relatively abrupt bends, than they are later in the horns. This may be due to crowding, as the spores after being released from the horn and when floating free tend to become less bent or crooked. The spores usually have only a slight curvature, which is ordinarily apparent in only one plane, but the actual shape is more nearly that of a greatly stretched spiral.

STRUCTURE OF SPORES

The spore as observed in the bright field shows more or less conspicuous septations (p. 553) and guttulae (p. 554) as commonly described in the literature for nearly all species of *Septoria*. The spores stain readily for bright-field observation, with accentuation of the septations and some changes in the appearance of the guttulae. One of the most

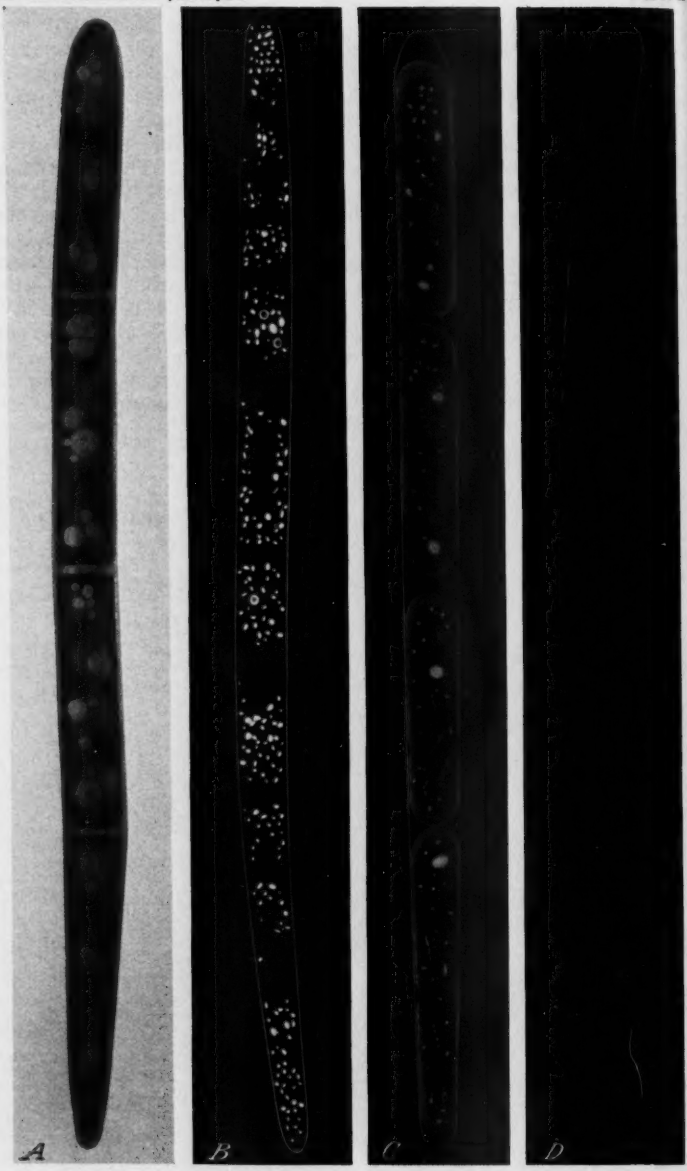
suitable quick-acting stains is cotton blue. Dark-field observation was much more satisfactory in revealing the details of spore structure. The spores were commonly observed in the uninjured, unstained condition, wherein certain activities could be observed within the spore. The mature *Septoria* spore has an integument, which is regarded as the spore wall and which encloses four separate protoplasts, each surrounded by an exceedingly thin, flexible membrane. The spore is, therefore, four-celled. The integument is a thin, homogeneous membrane, without visible surface markings or structures. The inner surface is likewise smooth. It appears to be permeable, as stain and solutions pass through it readily and react with the protoplasts within but have little or no effect on the integument itself.

A number of methods were tried to stain the spore wall, including the techniques of Coleman (*4*)[2], Wisselingh (*15*) and others, as well as the methods used with chitin and hemicellulose. None gave satisfaction. The most successful stain was obtained by the following procedure. A clean segment of freshly exuded spore horn was placed in water on a clean glass slide, the spores were spread well, and the slide was dried very slowly and lightly in the flame, thus fixing the spores to the slide. The spot of spores was then covered with a 10-percent aqueous solution of potassium hydroxide, and the slide was warmed for a few minutes. The hydroxide was rinsed off with water, the spot was covered with a saturated aqueous solution of picric acid, and the slide was warmed again for a few minutes. The picric acid solution was rinsed off, and the stained spores were mounted in water under a cover glass. This treatment stained the wall a lemon-yellow color.

Plate 1, *A*, represents a mature unstained spore, as seen in the bright field. It shows the unequal density of the protoplasm of the spore and the true guttulae as spots or globules of variable size of light-colored or relatively transparent material. The alleged cross walls are distinct and appear to be continuous throughout with the outside enclosing wall. The outside enclosing wall has a substantial thickness, which is uniform over the length of the spore. The spore as represented shows that the bending is approximately at the points of septation; the reason for this will appear later (p. 551).

In the dark field of the microscope the normal unstained mature spore shows other characteristics (pl. 1, *B*). The spore wall is reduced to a thin line, and what appear to be cross walls in plate 1, *A*, have lost their identity. The guttulae no longer appear as well-defined spots. The four protoplasts may be distinguished from each other by the enclosing membranes of the separate protoplasts, but in the normal state their membranes are seldom conspicuous and are not seen in every spore. The contents of the protoplasts resolve into a colorless fluidlike medium in which there are small particles or granules shining in the reflected light. The particles tend to assemble in groups, usually separate from each other, the particles in a group not being agglutinated. A few particles are much larger than others, and the light reflected from the equatorial portion causes them to appear as rings. These may be some of the more darkly shaded portions of the bright-field drawing. Many of the bright particles of the protoplast are in fairly rapid motion, which is of the nature of

[2] Italic numbers in parentheses refer to Literature Cited, p. 559.



Spores of *Septoria apii-graveolentis:* *A*, Mature unstained spore as seen in the bright field of the microscope; *B*, mature unstained spore as seen in the dark field; *C*, mature spore plasmolyzed by heating and stained with basic fuchsin as seen in the dark field; and *D*, ruptured, living, unstained spore in which germination has begun, as seen in the dark field.

Brownian movement, though other closely adjacent particles in the same group are relatively or wholly quiet.

Small particles are observed to rotate in a circle of small radius, and then suddenly to move in a more or less straight line to another part of the protoplast as much as a third or a half the length of the protoplast away, where the rotating motion begins again. The particle then returns to its former position and repeats the action indefinitely and with some regularity. There is no streaming movement of the protoplasmic contents. The grouping of the particles in the protoplast is such that there are often single spots as large as 15 to 25 percent of the volume of the protoplast that are free from particles, except as some may be moving across it. The groups of particles tend to settle toward the ends of the protoplasts, though a more general distribution is frequent. In no protoplast, stained or unstained, has there been observed any structure that could be construed as a nucleus. The areas within the protoplasts that are relatively free of particles permit the light of the bright field to pass through them with little interference and are the large guttulae so conspicuous in some species of *Septoria*.

The position, size, and distribution of the particles within the protoplasts in the dark-field view of the mature spore shown in plate 1, *B*, are characteristic of the unstained and uninjured spore. The points of septation are revealed very faintly at the abutment of the protoplasts at each end, but the point of septation does not show at the center. Though commonly absent, the cross member when present varies in appearance from spore to spore, depending on the position of the membranes relative to the source of illumination. When the protoplast particles are of sufficient size they are revealed as ringlike structures. Large particles that reflect wholly are either misshapen globules or material of a different sort.

The spores were treated with various solutions of sugar, salt, and other chemicals to induce plasmolysis, but without success. The cells appeared to be wholly permeable to such solutions. By carefully heating and drying the spores on a glass slide, the protoplasts can be shrunk so that they will draw away from each other and from the spore wall. Plate 1, *C*, represents a spore in which the contents were shrunk by heating and stained with basic fuchsin. The integument is revealed merely as a case that encloses the four protoplasts or cells. The protoplasts are detached from it, except as a mere matter of contact, and the integument appears to serve merely as a confining and protective covering. It is exceedingly thin—of the relative thickness illustrated in plate 1, *B* and *C*. The protoplasts have a membrane covering, which separates the contents of one cell from those of the next and also from the spore wall. The light reflected from the surface of the protoplast reveals a smooth, unmarred surface of no measurable thickness. The septa or cross walls that showed in the spore in the bright field have entirely vanished. In other words, there are no cross walls.

In the normal spore the protoplasts assume the shape of the integument and together completely fill it. Generally they abut at right angles to the spore wall, and so flexible is the membrane that the space is completely filled, as in plate 1, *B*. Nothing separates the protoplasts, and they lie end to end within the integument. The

transmitted light of the bright field passing through the area of contact of two protoplast membranes resolves itself into the septum or cross wall. The bending of the spore at the points of septation as shown in plate 1, *A*, is explained as follows: The pressure exerted by the turgidity of the protoplasts tends to stretch the integument. As the point of contact of the protoplasts, i. e., the so-called septum, is the most flexible in the spore, adjustments necessary in any spore under tension or pressure are best accommodated at these points. After the spore has been released from the confines of the pycnidium or horn its shape appears to depend on the integument. With the internal pressure relieved, as in plate 1, *C*, the tendency to curvature or bending is greatly lessened.

Plate 1, *D*, represents a spore, alive and beginning to germinate, that for some unknown reason has become ruptured. The integument has a definite break in the wall at one end. The protoplast adjacent to that end has also ruptured and collapsed, drawing the visible particle contents together within the shrunken membrane. The rest of the protoplasts, however, are alive, functioning normally, and growing. The live cell beyond the collapsed cell, released from the restraining pressure of the collapsed cell, has stretched out into the empty space without rupture or any apparent hindrance to its normal functioning. The single protoplasts seem to be units that function apart from the fate of the other units within the spore, though they commonly act in unison in germination and growth.

GROWTH AND GERMINATION OF SPORES

The *Septoria* spores from fresh celery material are not of uniform length, though at any single stage in their development they are relatively uniform in condition and aspect. The average length of 50 spores taken from a mature-appearing pycnidium examined within an hour after collection was 36.8μ. The spores were all three-septate and apparently wholly normal. An examination was made of 20 spores from a freshly exuded cirrus, and the same spores were followed through some successive germination stages. A suitable medium for the study of this process is celery juice, freshly extracted from petioles, filtered through paper, and used without sterilization. The slides were prepared by putting a segment of the cirrus in a drop of such juice, using a portion of the suspension as a hanging drop, and sealing and fastening the cover with rubber cement. The slides were held at room temperature, about 20° C. Instead of taking a large number of slides for observation, it was found best to use a single slide in which the conditions were uniform for all the spores under examination. As stated previously, the same 20 spores were observed throughout the period of observation, but they were not all the spores on the slide. Their average length when taken from the cirrus at the beginning of the period was 44.5μ. At the end of 4 hours their average length had increased to 50.4μ, with such a small corresponding increase in width that it could not be measured. In 5 hours the spores averaged 57.0μ in length, and in 6 hours they averaged 61.2μ.

During the next 2 hours there was no appreciable change in length, as the spores still averaged 61.2μ at the end of 8 hours. However, definite changes were then beginning. The spores were becoming

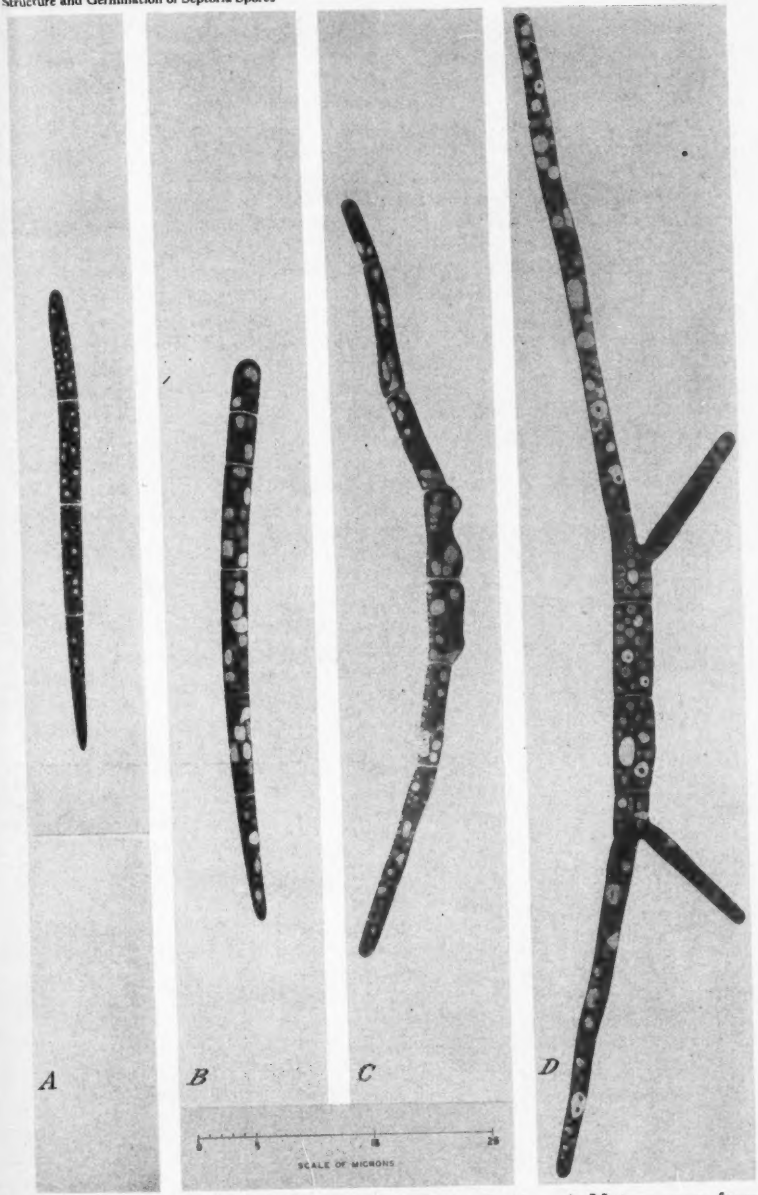
more crooked even at the end of 6 hours. Some were longer than others, but the shorter ones were swelling proportionately more than the longer ones. The swelling occurred over about one-half of the length. The contents of the spores seemed to be dividing into more protoplasts, as an increasing number of cells were apparent. At the end of 8 hours the increased diameter of the spores was very evident, bulges were appearing in many cells, and the protoplasts were more coarsely vacuolated than they were previously. At the end of 10 hours there were still more noticeable increases in diameters, and the swelling of the cells had become more pronounced. The protoplasts were separated slightly, and the central cells had become more heavily vacuolated. At this time the length averaged 62.3μ. During the next 2 hours growth was resumed, and at the end of that time the spores had reached an average length of 69.6μ. Branching was beginning, and it was evident that the spores had now germinated. The spore lengths of the single observation are summarized in table 1. Other series of observations on similar spore material gave like, though not identical, results.

TABLE 1.—*Length of spores of Septoria apii-graveolentis after different periods in celery juice*

Source	Number	Period	Length
		Hours	μ
Pycnidium	50	0	36.8
Cirrus	20	0	44.5
		4	50.4
		5	57.0
		6	61.2
		8	61.2
		10	62.3
		12	69.6

Another series of slides was prepared for germination study, with raw celery juice as a medium and with spores from a freshly exuded cirrus. Spores were selected for shorter length and were more nearly like those from a pycnidium. An attempt was made to promote germination as rapidly as possible. As the spores reached the end of the allotted periods they were flushed with a solution of cotton blue, which killed, fixed, and stained them and revealed the vacuolated structure in considerable detail. The spores were observed in the bright field of the microscope. The group (pl. 2) may be regarded as stages in the germination of a hypothetical *Septoria* spore, which may be considered as typical. The first spore, killed immediately, is shown in plate 2, *A*. The spore is 38μ long, slightly over 1.5μ wide at the widest point, and well filled with protoplasm staining to appear as finely divided, foamlike globules, some brighter than others. Brightness, however, may be a matter of position, as the larger particles do not appear to differ from the smaller except in size. The dissepiments appear as definite and substantial septations, which are continuous with the outside wall.

A second spore originally of the same size and aspect as that of plate 2, *A*, killed and stained with cotton blue at the end of 4 hours, is shown in plate 2, *B*. The spore has undergone several changes. The length has increased to 46μ and the width at the widest part to



Stages in spore germination of *Septoria apii-graveolentis*. *A*, Mature spore from a freshly exuded cirrus. *B–D*, Germinating spores: *B*, at the end of 4 hours; *C*, at the end of 12 hours; *D*, at the end of 24 hours.

about 2.4μ. The two end cells have divided so that the spore is five-septate, and the tapered end of the spore has increased in length more than the other cells. The appearance of the spore contents has changed markedly from that of plate 2, *A*. There are larger globules of uniform-appearing material, the finely divided structure is evident in the background, and the shape and size of the larger particles suggest that they are due to a flowing together or agglutination of the smaller particles to form the larger. The contents appear to be similar throughout, and the undivided center cells show no distinction from the divided end cells.

Plate 2, *C*, represents a spore at the end of 12 hours. The length has increased to 62μ, and the greatest diameter is nearly 3.3μ. The two pairs of end cells of the type of spore shown in plate 2, *B*, have increased in length, and the ultimate cell on each end has divided again. The two center cells have shortened somewhat but have increased in diameter and apparently are under some pressure from within, as they are unequally bulged and extended. The aspect of the contents is not markedly different from that of the spore in plate 2, *B*. The dissepiments appear to be heavier and wider, and the several cells, except the two end ones, are becoming more crooked and unequally stretched.

The comparative spore development at the end of 24 hours is represented by plate 2, *D*. The two center cells of the spore of plate 2, *C*, have here divided, and the outer cells of each division have sent out tubes similar in all respects to the growing end cells of plate 2, *C*. The center cells have grown slightly, the greatest diameter being about 3.75μ. The length of this spore from tip to tip is 95μ, though a median line would be slightly longer. The contents of the extended, slim ends are very highly elaborated, with larger guttulae than in the center cells. The dissepiments now appear to be true cross walls, and not the membranes of contiguous protoplasts, as in the original spores. From this stage the germinating spore merges into mycelium and its identity becomes lost. Under the conditions of the experiment the germination appeared to be retarded somewhat in the final stages, as though staleness of the medium, or byproducts, had checked the initial rate of growth.

MEANING OF APPLIED TERMS

In the routine examination of large numbers of *Septoria* spores from celery and other hosts, confusing variations have appeared in certain characters. As species of *Septoria* have been defined in part in terms of septation and guttulae, these structures are of prime interest and should be understood more clearly. Since the spore of the celery *Septoria* is characterized by certain features that apply throughout the genus *Septoria*, it is evident that the meaning of the terms applied to the spores needs to be evaluated.

SEPTATION

The spores of *Septoria* are commonly spoken of as "septate," but not always with a clear understanding of the implications of the word. A great variety of structures, also designated by such names as "dividing wall," "membrane," "partition," and "dissepiment," have been called "septum." Many of the authors of texts (as Owens, *11*) use the term

"cross walls," and in this they are justified because some mycologists—Ranojević (*13*), Petrak (*12*), Diedicke (*5*), and others—have used the term in their descriptions. In the case of *Septoria*, however, the term "cross wall" is incorrect since the connotation would justify the belief that the spores are chambered. "Septum" is not entirely free from this meaning, though it is subject to wide interpretation. The term "dissepiment," meaning a separating tissue, characterizes with some accuracy the structure that exists. Any term employed to mean a tissue, the function of which is to divide one part from another, is misused here. The tissues that separate the cells of the spore are the abutting plasma membranes of the cells; under natural circumstances these present the aspect of a single dissepiment. The term "septate," which is founded in the designation of the genus itself and cannot be abandoned, should be used, but with an understanding of the import of the word and its modification and application to special cases.

GUTTULA

The accepted meaning of "guttula" as a small drop-shaped spot, and "guttulate" as having drops or spots, is universally understood. The use of the term is very common throughout the literature of *Septoria*. A close study of a *Septoria* spore reveals some confusion as to the nature of the guttula, especially when it is realized that with many species this structure occupies an important part of the spore description. Stained, the spores reveal a great many guttulae, and these are correctly named in that they are small spots or droplets of protoplasmic origin or substance. But these are not the usual guttulae of the mycologists. The guttulae of the descriptive literature are bright spots caused by the transmitted light in the microscope passing through the clear areas of the cells of the spore, which, because of structural advantages, intensify the light as do small lenses. Such clear spots are shown in plate 1, *B*. The single cells may have a single bright spot, or they may have two, but the authors have observed no spores of any sort in which this feature was constant within a species as a single bright spot per cell. To base specific differences on this feature does not seem justified considering its nature and the likelihood that these spots are duplicated with equal variation in a great many species. To regard them as spots is justified, but to consider them as drops is not, and for that reason the term "guttulate" should be used with some reservation. To designate the places as "bright" or "lustrous" spots would be more nearly descriptive, though it does not seem to be possible to forego the use of the anomalous term "guttulate."

MATURITY

There seems not to be any special definition of the term "maturity" as applying to fungi, though in most cases it is assumed to mean that the fungus structure is capable of fulfilling its physiological function and can attain its functional destiny in the proper environment. This may apply to many fungus structures, such as pycnidia, perithecia, spores of all kinds, and even conidiophores and other parts. A mature fungus spore is a reproductive entity that has terminated physiological conjunction with the thallus that produced it and is capable of germination and growth in the proper environment.

The *Septoria apii-graveolentis* spore is borne on a conidiophore, first as a single-celled body; then with the formation of a single septum it becomes two-celled. The two cells divide simultaneously, so that the spore has three septa and four cells. When the spore attains three septa and four protoplasts or cells it has reached mature form, but it may or may not leave the pycnidium at this time. Until the spore is three-septate it is immature. Spores that have more than three septa have begun to grow and will germinate very soon unless growth is suspended. The interval between maturity and growth may be very short. Many spores appear to begin growth and then to suspend further activity. This is the reason that five- and seven-septate spores are sometimes found. This growth may occur while the spores are yet held in the cirrus, or a very few may be so found while in the pycnidium. There should be no spores with an even number of septa, as the manner of cell increase obviates them. The spore is mature when it becomes three-septate, and it is free to leave the pycnidium when environmental conditions permit.

A GENERAL CONSIDERATION OF SEPTORIA SPECIES

It is evident from the published descriptions of species of the genus *Septoria* as given by Saccardo (*14*) that the authors were generally uncertain regarding the septation of the spores. It is frequently impossible to compare specimens with published descriptions and to arrive at definite conclusions as to their identity, largely because of the different terms used in designating septation, or the wholly vague meaning of such terms. One finds such descriptive statements about spores as the following:

Term:	*Saccardo (14)*
Subcontinuis	3: 481 (sp. 36).
Obsolete guttulatis	3: 482 (sp. 37).
Obsoleteque septatis	3: 482 (sp. 42).
3–7 nucleolatis	3: 483 (sp. 48).
Obscure nucleolatis	3: 484 (sp. 53).
Obscure septatis	3: 485 (sp. 61).
Spurie 3–4-septatis	3: 489 (sp. 83).
Pluri-nucleolatis	3: 491 (sp. 96).
Obsolete guttulato-septulatis	3: 495 (sp. 117).
Multiguttulatis	3: 511 (sp. 212).
Indistincte nucleolatis	3: 518 (sp. 257).
Guttulatis vel septatis	3: 519 (sp. 263).
Continuis v. plasmate obsolete partitis	25: 410 (sp. 25).

The situation in the genus *Septoria* has long been one of confusion. Beach (*2*) concluded that in certain species morphological characters vary considerably under different environmental conditions and that the value of measurements now given in specific descriptions is questionable. Garman and Stevens (*7*) made a comprehensive study of the descriptions of *Septoria* species based on those of Saccardo (*14, v. 1–22*). They believed that spore length was an important clue to the proper placing of species. They summarized Saccardo's descriptions, giving, among other factors, the number of septa, number of guttulae, and spore shape for 1,181 species. To this summary of the statements on septation the authors have added a digest of 231 other species from Saccardo (*14, v. 25*) that have been classified in the manner of Garman and Stevens. This classification is shown in table 2.

TABLE 2.—*Supplemented summary of Garman and Stevens' classification of Saccardo's statements on septation in species of Septoria*

Citation	Classifier of statements	Species	Statements about septation			
			Absent	Indefinite	Definite	
					Septa present	Septa absent (sporescontinuous)
		Number	*Percent*	*Percent*	*Percent*	*Percent*
Saccardo (*14, v. 1–22*)	Garman and Stevens (7)	1,181	36.5	17.4	28.5	17.7
Saccardo (*14, v. 25*)	Authors	231	21.2	14.7	40.7	23.4
Total		1,412	34.0	16.9	30.5	18.6

Table 2 represents at best only an incomplete analysis of the septation statements found in the descriptions of *Septoria* as given by Saccardo (*14*). Approximately 34 percent of the descriptions have no reference to septation whatever, and 18.6 percent of the species are said to have continuous spores (septa absent); from these last no inferences are justified. Approximately 16.9 percent have indefinite statements about septation. Of the 30.5 percent that are described in terms of septation, a further analysis reveals that in the majority of cases the specifications are vague and possibly misleading, and nothing conclusive is warranted by further segregations. It may be doubted whether many of the species with one-septate and even-number-septate spores belong to *Septoria* at all, if the observations recorded herein are correct. Beach (*2*) found that septation appeared in some of the species with which he worked, when they were properly stained, but he did not always state how many septa were revealed. Hemmi (*9*), in describing *S. glycines*, found the spores to consist of "one to three, rarely four cells," which would indicate that they were continuous, one- or two-, and rarely three-septate, but that the septa might be easily overlooked. On germination the septa showed clearly. Wolf and Lehman (*16*), describing *S. glycines* Hemmi, illustrated one-, two-, and three-septate and continuous spores. Frank (*6*), studying *S. avenae*, found the spores to be two- to four-septate. The illustrations of *S. sisymbrii* given by Ranojević (*13*) show one-, two-, three-, four-, and five-septate spores, a condition that is untenable in the authors' understanding of the development of mature *Septoria* spores.

The description given by Saccardo (*14, v. 25, p. 454*) for *Septoria apii-graveolentis* contains no mention of spores whatever. Cochran (*3*) studied these spores with great care and found that the number of septa varied from zero to seven. The spores studied for this character were classified by Cochran as follows:

Number of septa per spore:	*Percent of spores*
Less than 3	10
3	60
4	18
5	10
More than 5	2

The 10 percent of the spores that had less than three septa undoubtedly were immature and may have consisted largely of one-septate spores, though what appeared to have been two-septate spores may have been observed. The three- and five-septate spores would be normal, especially as germination had evidently begun as shown by the presence of the five-septate and more-than-five-septate spores. The authors, however, cannot account for such a large proportion as 18 percent of four-septate spores.

DISCUSSION

The authors have examined a number of *Septoria* species from widely varied hosts and find that the odd-number-septate spores are characteristic throughout. The spores from *Septoria* species producing cirri are generally of larger size and may have a higher number of septa, as five and seven, and possibly more. Three is the number most commonly found. A few of the many species examined were *Septoria dulcamarae* Desm. on *Solanum dulcamara* L.; *S. solitaria* Ell and Ev. on *Rhododendron occidentale* Gray; *S. silenicola* Ell. and Mar. on *Silene gallica* L.; *S. alnifolia* Ell. and Ev. on *Alnus rhombifolia* Nutt. and on *A. rubra* Bong.; *S. scabiosicola* Desm. on *Scabiosa arvensis* L.; *S. aceris-macrophylli* Pk. on *Acer circinatum* Pursh; *S. angularis* Dearn. and Barth. on *Solidago latifolia* L.; *S. populi* Desm. on *Populus trichocarpa* Torr. and Gray; *S. rubi* Westd. on *Rubus vitifolius* Cham. and Schlecht.; *S. pentstemonicola* Ell. and Ev. on *Penstemon cordifolius* Benth.; *S. stachydis* Rob. and Desm. on *Stachys californica* Benth.; *S. scrophulariae* Pk. on *Scrophularia californica* Cham. and Schlecht.; and *S. corylina* Pk. on *Corylus californica* (A. DC.) Rose. *Septoria* on celery plant parts and seeds from many parts of the world was examined.

The examination of a number of fungi now known as species of *Septoria* indicates that those characterized by shorter and wider spores and with one or two septa are probably misclassified in many instances and should be critically examined for possible transfer to some other genus. Some of the species examined would more properly find their places in *Ascochyta*, *Diplodina*, *Hendersonia*, or elsewhere; and among the many-septate forms are those that may belong in *Stagonospora*, *Rhabdospora*, and possibly *Cylindrosporium*. As Garman and Stevens (*7*), Beach (*2*), and others have pointed out, the greatest confusion exists in the genus *Septoria* and the entire genus is in need of study and revision. As a mycological study, Grove's (*8*) treatment of the species of *Septoria* is the most adequate one so far. Diedicke (*5*) found cause to reclassify many species of *Septoria*, and a detailed study of the pycnidial formation and structure would be required to establish the proper relation of these species, as pointed out by Archer (*1*) in the case of other Sphaeropsidales.

The manner of septation in *Septoria apii-graveolentis* as the authors have found it and the uncertainty that results from observing these minute spores solely by means of the bright field of the microscope indicate that observation by the dark-field method is necessary for a reliable estimate of the number and kind of dissepiments in these spores. The customary manner of spore septation as it appears in

S. apii-graveolentis, by simultaneous division of the end cells after the formation of the primary septum, indicates that an even number of septa in these spores is anomalous. Though even-number-septate mature spores are commonly reported to be present, the authors have never observed one in this species. Other species also show the odd-number-septate structure consistently, though the dark field of the microscope usually has to be resorted to to make the odd-number septation distinguishable.

The spores of fungi within a species are generally very uniform in size, structure, and other characters, varying only in small percentages from the normal. Ranojević (*13*), however, credited *Septoria sisymbrii* with spores varying in length from 19μ to 62μ. Leonian (*10*) found that a higher food concentration in culture produces more numerous pycnidia and that rich hyphal growth and pycnidial development are parallel within a very wide range. From the authors' study of the celery *Septoria* it seems certain that the spores become mature on attaining the three-septate condition, but not before. It is possible that the phenomenon of suspended germination is common throughout the genus *Septoria* and forms the basis for the wide variation reported in the size and septation of the spores, multiseptation especially being an evidence of growth after maturity. From mycologists greater detail should be required in the definition of the conditions under which the fungus was found and the environment to which it was exposed. As no rest period is necessary, complete germination may occur promptly. This is evidently a factor in the spread of the disease caused by this fungus.

CONCLUSIONS AND SUMMARY

A detailed study has been made of the spores of *Septoria apii-graveolentis* Dorogin from cultivated and escaped celery. The structure of the spore and its growth and germination are given extended treatment. The meaning and significance of terms applied to the spore, such as "septation," "guttula," and "maturity," are analyzed.

Assuming that the spores of *S. apii-graveolentis* reveal the nature of *Septoria* spores in general, the writers have discussed the inadequacy and confusion existing in the descriptions of species in the genus *Septoria* and have offered suggestions for greater precision in description and care in observation.

The following conclusions have been reached:

(1) The spores of *Septoria apii-graveolentis* are mature when they consist of four cells, i. e., when they are three-septate.

(2) Septation in the spores of this fungus is caused by the abutting membranes of contiguous cells within the integument. There is no wall or true septum within the mature spore.

(3) The guttulae of the *Septoria* spores in descriptive mycological literature are bright spots, produced by lens action on the clear portions within the cells of the spore.

(4) Germination is accompanied by cell proliferation, usually by division of the end cells of the spore. There are changes in the appearance of the cell contents with progressive stages of germination.

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EXPERIMENTS WITH ARSENITE SPRAYS TO ERADICATE SCLEROTINIA LAXA IN STONE-FRUIT TREES AS A MEANS OF CONTROLLING THE BROWN ROT DISEASE IN BLOSSOMS [1]

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INTRODUCTION

The development of eradicant fungicides has received increased attention since Keitt and Palmiter (*15*) [2] described experiments conducted over a 13-year period (*8, 9, 10, 14, 16, 17, 18, 21*). According to these workers, the survival level of a fungus within the orchard is lowered by sprays applied at a vulnerable stage of its development. Thus the ascigerous stage of *Venturia inaequalis* proved vulnerable to a copper-lime-arsenite mixture applied to the leaves before they fell or after they were on the ground. The copper-lime-arsenite mixture was prepared by combining copper sulfate, lime, and monocalcium arsenite. In preliminary tests with other fungi, these writers found that this fungicide destroys such diverse parts as mycelia, conidia, pycnidia, exposed and embedded ascocarps, sclerotia, and basidiospores. Tims (*25*) furnishes additional evidence that it is effective against sclerotia of *Corticium stevensii* and *C. microsclerotia* on fig trees.

In 1939 Keitt (*11*) reported successfully using "sodium dinitro-o-cresol" (Elgetol) against the perithecial stage of *Venturia inaequalis*. Further evidence of its effectiveness against this fungus is reported by Keitt, Clayton, and Langford (*12*), Keitt, Clayton, and Moore (*13*), Palmiter (*20*), Sharvelle (*23*), and Kadow and Hopperstead (*7*). Anderson (*1*) found that it prevented ascospore development by *Coccomyces hiemalis* in sour cherry leaves, and Sharvelle (*23*) used it against *Plectodiscella veneta* on raspberry canes. Strong and Cation (*24*) report that "Elgetol regular" in 1-percent strength prevented the extension of the telial columns and killed teliospores of *Gymnosporangium globosum*, *G. juniperi-virginianae*, *G. clavipes*, and *G. clavariaeforme* in the galls on *Juniperus virginiana* and *J. communis*.

Various other materials apparently have some value as eradicants. In the small-scale trials of Holz (*4*), unoiled calcium cyanamide applied dry to apple leaves lying on soil prevented perithecial development of *Venturia inaequalis*. By dusting the orchard floor with this material, Huber and Baur (*5*) and Huber, Baur, and Breakey (*6*) destroyed apothecia of *Sclerotinia fructicola*. Hendrick (*2*) determined that urea, ammonium sulfate, ammonium chloride, ammonium carbonate, potassium sulfate, potassium chloride, and lime-sulfur had deleterious effects on the ascigerous stage of *V. inaequalis*. The successful use of paradichlorobenzene against the tobacco downy mildew fungus [3] by

[1] Received for publication May 13, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 593.

[3] No attempt is made here to review the numerous papers published on this subject. Angell, Hill, and Allen appear to have first used benzol for controlling tobacco downy mildew in Australia. The work in this country has been done by Wolf, McLean, Pinckard, Darkis, and Gross.

McLean and Pinckard (*19*) and by Pinckard (*22*) shows how the gaseous phase of a material may kill the fungus in living tissue without unduly injuring the host.

In 1938 and 1940 the writer and a coworker (*26, 27*) published the results of tests with calcium, zinc, and sodium arsenites, applied to apricot and almond trees to suppress the sporodochia of *Sclerotinia laxa* on blighted hold-over twigs. The present paper gives the details of these trials, together with results secured in 1940.

Certain other materials, such as Elgetol, paris green, and tar oil, were tested; but as further trials with these materials are necessary before conclusions can be drawn, the results are not reported here.

THE BROWN-ROT DISEASE OF STONE FRUITS

The California stone-fruit industry suffers severe losses from blossom and fruit infection caused by two species of *Sclerotinia*. Upon finding apothecia of *Sclerotinia fructicola* (Wint.) Rehm to be fairly abundant on peach mummies on the orchard floor, Hewitt and Leach (*3*) studied the distribution of this species and of *S. laxa* Aderh. and Ruhl. They determined that whereas *S. fructicola* was primarily responsible for peach-fruit rot, *S. laxa* was almost exclusively responsible for serious blossom blight in apricots and almonds.

Since none of the apothecia that Hewitt and Leach found were those of *Sclerotinia laxa*, their studies show, like those of others, that the perfect stage of this species if produced at all is exceedingly rare. The fungus (*S. laxa*) causing blossom infection in apricot and almonds overwinters in the twigs that are blighted after blossom infection, and (in apricot) on the occasional rotted fruit that hangs on the tree throughout the winter. Apparently, therefore, the cycle of development of *S. laxa* is confined to the trees. The primary inoculum for blossom infection is conidia produced on sporodochia, the spore-bearing cushions (fig. 1) which develop on twigs and mummied fruits during the winter. As fruit rotting by this fungus is comparatively uncommon in apricot and apparently unknown in almond, the major source of conidia is blighted twigs.

DEVELOPMENT OF SPORODOCHIA IN RELATION TO SEASON

After blossom infection in the spring, *Sclerotinia laxa* enters the small twigs and blights the portions distal to the point of entry. Conidia are produced on blighted blossom parts during early summer, but are less abundant in the late summer and autumn, and probably do not survive the winter. Sometime during the winter the sporodochia develop on the blighted twigs and blossoms, and immediately produce conidia (fig. 1). Observations on the period of winter during which the sporodochia develop are given below.

To judge from casual examinations made during the winter of 1937–38, sporodochial development began sometime in early February, none having been observed on almond trees in late January. During the winter of 1938–39, when more systematic observations were made, sporodochia were found on both almond and apricot twigs in late December. As figure 2 indicates, they became numerous on almond twigs between about December 27 and February 7, after which few or none appeared. In a prune orchard, on the other hand, they began to appear somewhat later and increased in number more slowly than in the almond orchard.

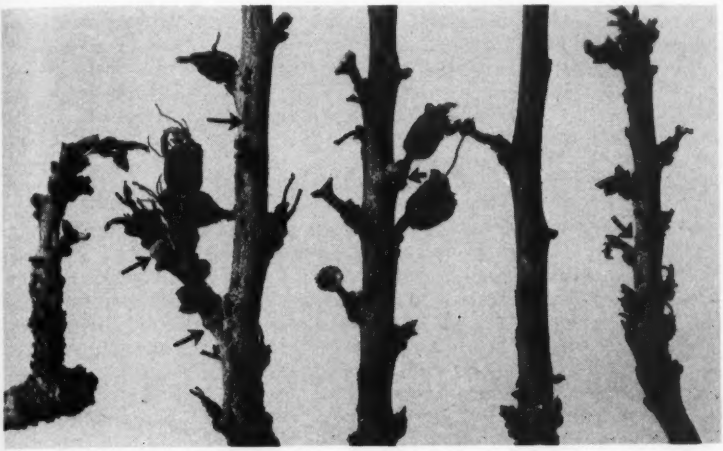

FIGURE 1.—Sporodochia (arrows) produced by *Sclerotinia laxa* on almond twigs in 1938. These structures, produced during the winter, bear conidia which are the only source of blossom infection the following spring. It is to prevent the development of these sporodochia, or to kill them (and the conidia thereon) after they develop, that eradicant sprays are applied in winter.

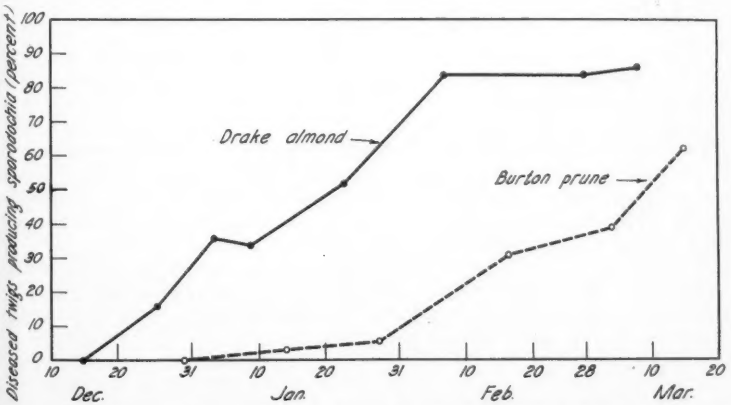

FIGURE 2.—Seasonal development of sporodochia during the winter of 1938–39 on blighted hold-over twigs in trees of Drake almond and Burton prune, as expressed by the percentage of blighted twigs bearing visible sporodochia at each succeeding date of examination.

Variations in the time sporodochia developed in different districts were marked in 1938–39. In the central valleys (Sacramento and San Joaquin) they developed on almonds and apricots over about the same period as indicated for almond in figure 2, but in coastal districts (Santa Clara and Pajaro Valleys) they appeared later.

In the winter of 1939–40 these structures did not begin to appear until late January and were not abundant until after the middle of February. As in 1938–39, they developed later in coastal counties than in the interior.

PRESENT CONTROL METHODS

Present measures for controlling the serious blossom blight of apricots and almonds consist in applying bordeaux mixture to the blossoms as they emerge from the bud scales in the spring To cover the rapidly expanding blossoms completely and to insure successful control, two sprays are frequently necessary—one immediately after blossoms emerge, and another when some of the blossoms are open. Reducing the hold-over sources of the fungus, by removing blighted twigs and mummied fruit from the tree, is recognized as a desirable supplementary procedure.

When conditions strongly favor development of the disease, as during the past 4 years, successful control is difficult for the following reasons: (1) Frequent rains in the spring may prevent application of the fungicide at the critical period; (2) the critical period in certain years is too short to permit coverage of large orchards; and (3) removal of all blighted twigs and mummied fruits from the trees is impractical and too expensive.

ELIMINATING THE SPORODOCHIAL STAGE WITH ERADICANT SPRAYS AS A MEANS OF CONTROLLING THE BROWN ROT DISEASE IN BLOSSOMS

The presence in the trees of the only known hold-over stage of *Sclerotinia laxa* would seem to afford an opportunity for attacking the fungus at a vulnerable point. Could this stage be eliminated or greatly reduced, the incidence of the disease should be lessened. The relatively inefficient method of removing blighted twigs and mummied fruits has, in certain instances, reduced the blossom blight; but in case of epidemic development the blighted twigs are too numerous to be removed. A spray capable of eliminating the sporodochial stage is, therefore, desirable. Whether it prevents development of the sporodochia or destroys them (and the conidia borne thereon) after they develop depends, of course, upon its properties and upon the time of application in relation to their initiation. In any event, the period of application would be sometime during the dormancy of the trees.

EFFECT OF ARSENITE SPRAYS ON THE DEVELOPMENT OF SPORODOCHIA

According to the observations reported earlier (fig. 2), the period of maximum sporodochial development, though varying somewhat from year to year and from district to district, extends from early January until late February. If, therefore, sprays should be applied before mid-January, or in some years before early February, few sporodochia would be present. To be of value at this time the spray

should be capable of preventing the development of sporodochia as well as killing the few already present.

This section reports the results of spraying with calcium, zinc, and sodium arsenites before sporodochia developed in large numbers. The effect of these materials on the fungus was gauged by determining the abundance of sporodochia on 100 to 150 blighted hold-over twigs, collected at random from each sprayed and unsprayed plot during the blossoming period or shortly after. In certain orchards where sporodochial development was followed closely, 25 twigs from each tree in the plot were examined. These examinations were repeated several times on some of the more important plots. To observe the sporodochia the twigs were held in a strong light under a hand lens.

According to observations made early in the work, the effect of the sprays on sporodochial development can be shown (1) by ascertaining the percentage of blighted hold-over twigs that bear sporodochia, and (2) by determining, on twigs that bear sporodochia, the average number of these structures per twig. As neither of these values alone gives the entire picture of sporodochial abundance, both are presented in the tables, together with their product as an index number. This index number should be regarded as evaluating the frequency of sporodochia on the samples collected and not as measuring the total present in the trees. To obtain a measure of the total present, one would have to determine the total number of hold-over twigs, a task for which no feasible procedure has been devised.

Spray Materials and Methods of Application

The monocalcium arsenite used (hereafter designated as calcium arsenite) was a finely ground powder containing 69 percent of arsenic as As_2O_3 and 22 per cent of As_2O_3 in water-soluble form.

The zinc arsenite (so-called buffered) was a finely ground powder containing 35 percent of As_2O_3 and 0.5 percent of water-soluble arsenic.

Solutions of sodium arsenite sold as weed killers were employed in most cases. The solutions were used at a rate which would be equivalent to 1, 2, or 3 pounds of sodium arsenite per 100 gallons of water.

Bordeaux mixture added to calcium and zinc arsenites was prepared from finely ground copper sulfate and hydrated lime. The calcium or zinc arsenite was added to the spray tank after the bordeaux was prepared.

The zinc sulfate-lime combination used with calcium arsenite in a few tests was prepared by dissolving the zinc sulfate in water in the spray tank and adding hydrated lime suspended in a small amount of water. The calcium arsenite was then added.

The oil added to the arsenites was a dormant type of petroleum emulsion having a 70 percent unsulfonated residue.

Other supplemental materials were potassium ethyl xanthate in powder form and Hydrolene No. 66, a powdered organic wetting agent. The procedure by which the latter material was combined with calcium arsenite in one test will be described at the proper place.

The sprays were applied as a fine mist to all twigs and branches, care being taken to avoid drenching. Except where noted, the spray dried thoroughly before rains occurred.

Comparison of the Effects of Different Concentrations of Calcium Arsenite

Five Drake almond trees sprayed on January 25, 1938, with calcium arsenite, 4 pounds per 100 gallons, were examined on February 17. Between these dates numerous sporodochia developed in unsprayed trees, but few developed in the sprayed trees (table 1). Later examinations, moreover, revealed no further development of these structures in sprayed trees, though a measurable increase occurred in unsprayed trees.

The following winter, 1938–39, 1, 2, and 4 pounds of calcium arsenite per 100 gallons of water were applied in a number of apricot and almond orchards and in one prune orchard (tables 2 and 3 and fig. 3).

Table 1.—*Development of sporodochia and of blossom blight in almond trees sprayed with calcium arsenite, zinc arsenite, alone and with oil, and sodium arsenite, 1938*

Treatment [1] (Pounds per 100 gallons)	Trees observed	Twigs with sporodochia (A)	Average number of sporodochia per twig [2] (B)	Index number (A×B)	Blossoming twigs blighted [3]
	Number	*Percent*			*Percent*
Unsprayed	7	69	5.4	373	62
Calcium arsenite 4–100	5	12	1.4	17	14
Calcium arsenite 4–100+4 percent oil	5	3	1.0	3	13
Zinc arsenite 4–100	4	22	2.1	46	30
Zinc arsenite 4–100+4 percent oil	3	35	2.1	74	27
Sodium arsenite 1.2–100	2	50	5.0	250	52

[1] Sprays were applied Jan. 25 before sporodochia developed.
[2] Observations on sporodochial development were made Feb. 17. These values were obtained by dividing the total number of sporodochia on the samples by the number of twigs which produced sporodochia.
[3] Disease data were taken after twigs which bore infected blossoms were blighted.

Table 2.—*Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with three concentrations of calcium arsenite used alone and in combination with other materials, December 1938 and January 1939*

APRICOT

Orchard No.[1]	Date sprayed	Treatment (pounds per 100 gallons)	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
11	Dec. 5–8	Unsprayed	79	9.8	774	29.0
		Calcium arsenite 4–100	2	1.0	2	.3
		Calcium arsenite 4–100+4 percent oil	3	1.0	3	.1
		Calcium arsenite 4–100+2 percent oil	5	1.0	5	.2
		Calcium arsenite 4–100+bordeaux mixture 8–4–100	4	1.0	4	.1
		Calcium arsenite 2–100	7	2.7	19	.2
		Calcium arsenite 2–100+2 percent oil	3	1.3	4	.5
		Calcium arsenite 2–100+bordeaux mixture 8–4–100	4	2.0	8	.2
		Calcium arsenite 1–100	16	2.6	42	.2
		Calcium arsenite 1–100+2 percent oil	12	2.4	29	.2

[1] Locations of the orchards were as follows: 11 and 11A, Contra Costa County; 14, Sacramento County; 15, Monterey County.

TABLE 2.—*Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with three concentrations of calcium arsenite used alone and in combination with other materials, December 1938 and January 1939*—Continued

APRICOT—Continued

Orchard No.	Date sprayed	Treatment (pounds per 100 gallons)	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
		Calcium arsenite 1-100+bordeaux mixture 8-4-100.	17	2.1	36	0 3
		Calcium arsenite 4-100	3	2.3	7	.3
		Calcium arsenite 4-100+2 percent oil	2	1.0	2	.2
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	7	1.1	8	.1
		Calcium arsenite 2-100	6	1.2	7	.1
		Calcium arsenite 2-100+2 percent oil	3	1.3	4	.2
11	Jan. 18-19	Calcium arsenite 2-100+2 percent oil (120-tree plot).[1]	4	1.1	4	.4
		Calcium arsenite 2-100+bordeaux mixture 8-4-100.	6	1.7	10	.3
		Calcium arsenite 2-100+bordeaux mixture 8-4-100 (120-tree plot).[1]	9	1.9	17	.6
		Calcium arsenite 1-100	7	4.0	28	1.2
		Calcium arsenite 1-100+2 percent oil	7	2.4	17	.3
		Calcium arsenite 1-100+bordeaux mixture 8-4-100.	16	2.0	32	3.0
		Unsprayed	68	6.9	469	45
	Dec. 27	Calcium arsenite 4-100	3	1.3	4	2
		Calcium arsenite 4-100+2 percent oil	3	1.4	4	2
11A		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	5	1.3	7	2
	Jan. 18	Calcium arsenite 4-100+1 percent oil	2	1.5	3	3
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	4	1.6	6	3
		Unsprayed	79	10.1	798	.....
	Dec. 15-16	Calcium arsenite 4-100	4	1.0	4	.....
		Calcium arsenite 4-100+2 percent oil	1	1.0	1	.....
		Calcium arsenite 2-100	13	3.3	43	.....
		Calcium arsenite 2-100+2 percent oil	15	3.1	47	.....
15		Calcium arsenite 1-100	39	3.3	129	.....
		Calcium arsenite 4-100	7	1.0	7	.....
	Jan. 30-31	Calcium arsenite 4-100+4 percent oil	2	1.5	3	.....
		Calcium arsenite 2-100	5	1.4	7	.....
		Calcium arsenite 2-100+4 percent oil	5	2.1	11	.....
		Calcium arsenite 1-100	11	2.1	23	.....

ALMOND

		Unsprayed	85	6.8	578	33
		Calcium arsenite 4-100	2	1.0	2	3
		Calcium arsenite 4-100+2 percent oil	3	2.4	7	3
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	4	3.3	13	8
	Dec. 13	Calcium arsenite 2-100	1	1.1	1	4
		Calcium arsenite 2-100+2 percent oil	4	1.1	4	7
		Calcium arsenite 2-100+bordeaux mixture 8-4-100.	22	3.1	68	20
14		Calcium arsenite 1-100	18	2.9	52	9
		Calcium arsenite 1-100+2 percent oil	15	3.1	47	10
		Calcium arsenite 1-100+bordeaux mixture 8-4-100.	63	3.3	208	35
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	4	1.5	6	3
		Calcium arsenite 2-100	7	1.9	13	6
	Jan. 17	Calcium arsenite 2-100+2 percent oil	3	1.0	3	4
		Calcium arsenite 2-100+bordeaux mixture 8-4-100.	17	2.9	49	6
		Calcium arsenite 1-100+2 percent oil	17	2.2	37	11

[1] The 120-tree plots were sprayed with a large spray rig operating at 400 to 500 pounds pressure. Other plots were sprayed with a small rig operating at 275 pounds' pressure.

TABLE 3.—*Development of sporodochia and of blossom blight in apricot and in prune trees in different orchards sprayed at different dates with four concentrations of calcium arsenite, used alone and in combination with other materials, 1938–39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
Tehama	1; apricot	Unsprayed	49	4.9	240	39
		Calcium arsenite 4-100, Dec. 29	12	3.5	41	17
		Calcium arsenite 4-100+2 percent oil, Dec. 29.	8	2.7	22	10
Stanislaus	5; apricot	Unsprayed	70	9.4	658	46
		Calcium arsenite 2-100+2 percent oil, Jan. 20.	13	2.8	36	16
	12; apricot	Unsprayed	45	4.8	216	72
		Calcium arsenite 4-100+2 percent oil, Jan. 19.	1	1.0	1	65
		Calcium arsenite 2-100+2 percent oil, Jan. 19.	5	1.0	5	74
San Joaquin	9; apricot	Unsprayed	49	3.9	191	76
		Calcium arsenite 4-100, Nov. 30	3	1.0	3	49
		Calcium arsenite 4-100+bordeaux mixture 10-10-100, Nov. 30.	18	1.6	29	71
		Calcium arsenite 4-100, Nov. 30; sodium arsenite 1-100, Feb. 17.	----------	----------	----------	40
		Calcium arsenite 4-100, Jan. 19	4	1.5	6	38
Yolo	13; prune	Unsprayed	68	4.7	320	----------
		Calcium arsenite 4-100+2 percent oil, Dec. 12.	6	1.2	7	----------

That sporodochia developed more numerously in some orchards than in others is seen from variations in the index numbers of unsprayed plots. Unlike the 1940 results to be reviewed later, the efficacy of a given treatment did not appear to be influenced by the intensity of sporodochial development. Calcium arsenite 4–100, for example, was applied in 8 orchards and calcium arsenite 4–100 plus oil emulsion in 7. The sporodochial index numbers of untreated plots varied from a low of 191 to a high of 798, yet these treatments were no less effective at the higher than at the lower levels. Comparisons of the 1939 results secured in different orchards, therefore, do not require that the indices of treated plots be referred to the unsprayed indices, unless the percentage reduction in sporodochial numbers is desired.

At the rate of 4 pounds per 100 gallons of water, calcium arsenite was highly efficient in reducing sporodochial development; rates of 2–100 and 1–100 were less efficient. As compared with the check, however, the 1–100 concentration when used alone reduced these structures on an average 92 percent; the 2–100 concentration 97 percent. Between orchards, however, the 1–100 concentration varied more in effectiveness than the 4–100 concentration; obviously it was too weak for consistent results. The 2–100 concentration was decidedly more efficient; and since, as noted later, it injured the trees less than the 4–100, it seemed a promising strength for further trials.

Attention was paid particularly to the effect of calcium arsenite on the development of sporodochia in the cankers that formed on large limbs of almonds. Because of the thick and roughened periderm on large limbs, the suppression of sporodochia in cankers formed thereon

is presumably more difficult than on smaller twigs. Concentrations of 2–100 and above appeared, however, to prevent sporodochial development on these cankers as effectively as on small twigs.

On the basis of the 1939 results, 2 to 4 pounds of calcium arsenite per 100 gallons of water appeared to be the most promising concentrations. Because of injury by the latter strength, most of the 1940 treatments were given with 2 and 3 pounds per 100 gallons. Almonds had proved so sensitive to arsenites that concentrations greater than 2–100 were not used in 1940.

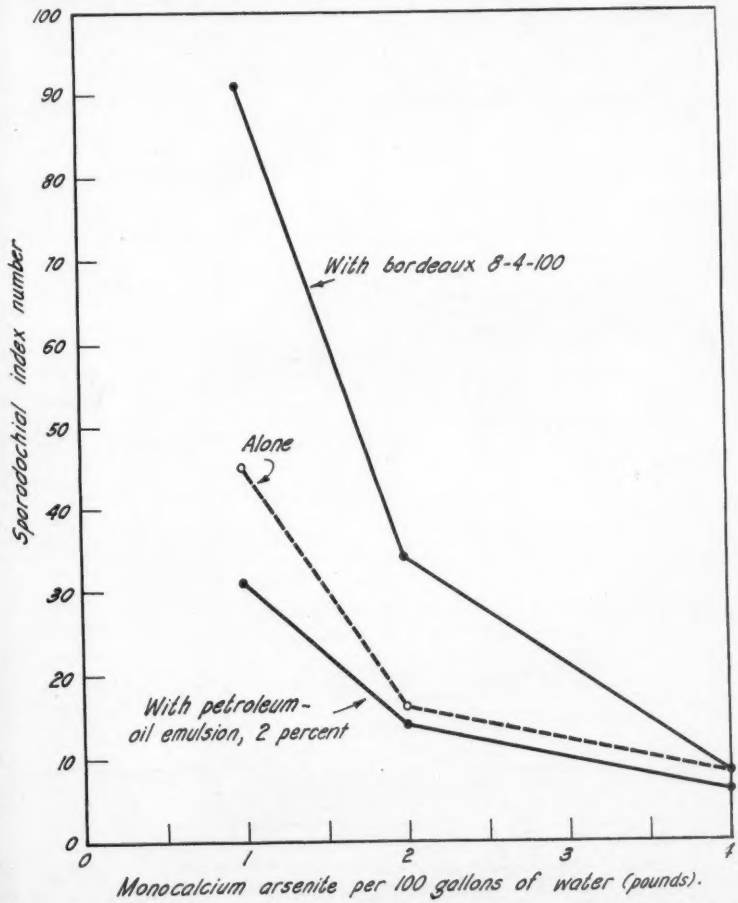

FIGURE 3.—Effect on sporodochial development of three concentrations of calcium arsenite used alone and in combination with petroleum-oil-emulsion and bordeaux mixture. As the sporodochial index numbers were obtained by multiplying the percentage of hold-over twigs with sporodochia by the average number of sporodochia per twig, the higher the index the lower is the efficacy of the spray.

In 1940 four varieties of plums—Wickson, Formosa, Sharkey, and Kelsey—were sprayed with calcium arsenite at the rate of 2–100 and 1–100; but because of insufficient holdover sources, results on sporodochial suppression were obtained only with Wickson.

Development of sporodochia began so late in the winter of 1939–40 that practically all applications were made before these structures developed in numbers. The details regarding dates of spraying, together with the results on sporodochial suppression, appear in tables 4 and 5. Before discussing the results, however, a word is necessary about the effect of the sporodochial level on efficiency of supression.

TABLE 4.—*Development of sporodochia and of blossom blight in apricot and in plum trees in different orchards sprayed at different dates with various concentrations of calcium arsenite used alone and in combination with other materials, 1940*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming shoots blighted
			Percent			*Percent*
Contra Costa	1; apricot	Unsprayed	51	6.8	347	39
		Calcium arsenite 2–100, Jan. 16	1	1.0	1	6
	2; apricot	Unsprayed	62	2.8	174	61
		Calcium arsenite 3–100, Jan. 13	8	1.5	12	2
		Calcium arsenite 2–100, Jan. 13	12	1.3	16	3
	3; apricot	Unsprayed	53	6.4	339	49
		Calcium arsenite 2–100, Feb. 9	26	3.3	86	1
Sacramento	4; apricot	Unsprayed	51	4.5	230	18
		Calcium arsenite 3–100+1 percent oil, Feb. 1.	6	2.0	12	2
		Calcium arsenite 2–100+1 percent oil, Feb. 1.	10	2.2	22	5
	5; plums	Unsprayed	53	3.5	186	26
		Calcium arsenite 2–100, Jan. 24	7	1.7	12	4
		Calcium arsenite 2–100+bordeaux mixture 4–2–100, Jan. 24.	19	2.3	44	6
		Calcium arsenite 1–100, Jan. 24	23	2.6	60	13
Tehama	6; apricot [1]	Unsprayed	43	5.7	245	76
		Calcium arsenite 3–100, Jan. 22	1	3.4	3	7
	7; apricot [1]	Unsprayed	31	5.0	155	37
		Calcium arsenite 3–100, Jan. 10	2	1.0	2	8
Stanislaus	8; apricot [1]	Unsprayed	67	5.2	348	22
		Calcium arsenite 2½–100, Jan. 22	12	2.2	26	4
	9; apricot [1]	Unsprayed	65	5.3	344	20
		Calcium arsenite 2½–100, Jan. 18	19	2.3	44	11
Ventura	10; apricot [1]	Unsprayed	25	8.3	208	35
		Calcium arsenite 2–100, Feb. 7	4	1.5	6	4
	11; apricot [1]	Unsprayed	64	3.3	211	20
		Calcium arsenite 2–100, Feb. 8	12	1.4	17	9
Alameda	12; apricot [1]	Unsprayed	55	7.9	435	25
		Calcium arsenite 3¾–100, Jan. 23	16	3.5	56	4

[1] Because of sparse blossom development in these orchards, data on the disease were secured by counting the number of blighted twigs on 5 uniform-sized branches in each tree.

TABLE 5.—*Development of sporodochia in almond and in apricot trees in different orchards sprayed at different dates with various concentrations of calcium arsenite used alone and in combination with other materials, 1940*

ALMOND

County	Orchard No.	Treatment (pounds per 100 gallons) and date sprayed [1]	Plots in different orchards	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A × B)
			Number	*Percent*		
Placer	13	Unsprayed	1	69	8.8	607
		Calcium arsenite 2–100 + bordeaux mixture 4–2–100, Feb. 1.	1	5	3.3	17
		Calcium arsenite 2–100 + potassium ethyl xanthate 5–100, Jan. 30.	1	9	2.0	18
Sacramento	41	Unsprayed	1	58	4.5	261
		Calcium arsenite 2–100, Jan. 16	1	13	3.1	40
		Calcium arsenite 2–100 + zinc sulfate-lime 4–2–100, Jan. 16.	1	15	3.0	45
		Calcium arsenite 2–100 + Hydrolene No. 66,[2] Jan. 16.	1	14	2.4	34
		Calcium arsenite 1–100 1 percent oil, Jan. 16.	1	16	2.8	45
	15	Unsprayed	1	78	6.8	530
		Calcium arsenite 2–100, Jan. 15	1	9	2.7	24
		Calcium arsenite 1–100 + 1 percent oil, Jan. 15.	1	16	2.7	43
San Joaquin	16	Unsprayed	1	70	6.3	441
		Calcium arsenite 1–100, Jan. 22	1	17	2.9	49
		Calcium arsenite ½–100, Jan. 22	1	20	4.9	98
	17	Unsprayed	1	39	4.6	179
		Calcium arsenite 1–100, Jan. 20	1	26	4.3	112
	18	Unsprayed	1	25	3.3	83
		Calcium arsenite 1–100, Jan. 15	1	7	2.0	14
	19	Unsprayed	1	26	5.2	135
		Calcium arsenite 1–100, Jan. 18	1	14	5.5	77
Stanislaus	20	Unsprayed	1	71	6.6	469
		Calcium arsenite 2½–100, Jan. 18	1	15	3.8	57

APRICOT

San Joaquin	21–27	Unsprayed	5	66	3.7	244
		Calcium arsenite 4–100	5	6	2.4	14
		Unsprayed	2	74	4.6	340
		Calcium arsenite 3–100	2	14	2.8	39
		Unsprayed	1	92	3.5	322
		Calcium arsenite 3–100 + bordeaux mixture 10–10–100.	1	48	2.1	101
Santa Cruz	28	Unsprayed	1	65	7.2	468
		Calcium arsenite 3–100	1	48	3.8	182
San Benito	29	Unsprayed	3	32	5.7	182
		Calcium arsenite 3–100	3	1	1.3	1
Alameda	30	Unsprayed	2	53	6.8	360
		Calcium arsenite 2½–100	2	12	3.2	38
		Calcium arsenite 2–100	2	23	5.2	120
Stanislaus	31	Unsprayed	2	51	4.2	214
		Calcium arsenite 2½–100	2	11	2.2	24
Solano	32	Unsprayed	1	67	4.5	302
		Calcium arsenite 2½–100	1	24	2.6	62
		Calcium arsenite 2–100	1	19	3.4	65
Monterey	33	Unsprayed	4	55	4.9	270
		Calcium arsenite 3–100	4	11	2.8	31

[1] The date of application differed among the apricot orchards listed in this table. Except in Solano County, where spraying was done on February 9, applications in the apricot orchards were between January 15 and 22.

[2] Hydrolene No. 66, an organic wetting agent, was added to calcium arsenite, with a little water, in the proportions of 1–5. The mixture was boiled for 30 minutes, dried, and ground to a fine powder. The powder was suspended in water to give arsenite equivalent to 2–100 calcium arsenite.

The inoculum level, as expressed by the sporodochia indices of unsprayed plots (tables 4 and 5), varied greatly in the different orchards. In order, therefore, to evaluate properly the results obtained with various concentrations of calcium arsenite, we must determine whether a given concentration reduced sporodochial development as effectively at the higher levels as at lower ones. When index numbers of individual treatments are assembled and plotted against increasing values of their check index numbers, the results are a series of curves with definite upward trends. That is, in a number of orchards where sporodochia developed in various degrees of abundance, suppression by a given calcium arsenite concentration decreased somewhat with increasing abundance of sporodochia. Under such circumstances, therefore, the suppressive efficacy of the various concentrations will be more accurately expressed by referring treatment index numbers to their check index numbers. A satisfactory method is to divide the treatment index number into the check index number. The primary purpose, however, that of comparing 3–100 and 2–100 calcium arsenite, can be served by plotting one against the other, placing increasing values of check index numbers along the horizontal scale.

The curves in figure 4 are obtained by grouping the control index numbers into a few large classes and averaging the treatment index numbers falling into each class, thus minimizing minor variations. According to these data, 3–100 calcium arsenite is somewhat more effective than 2–100, particularly where sporodochial abundance is greatest. Plots receiving 2½–100 were not numerous enough to be included in the figure, but the results on the whole were about the same as for 2–100.

Calcium arsenite 4–100 in five trials in San Joaquin county apricot orchards (table 5) consistently suppressed sporodochial development to a very high degree, the average reduction being 94 percent. It is noteworthy, however, that 3–100 and 2–100 concentrations, applied in other apricot orchards where the sporodochial level was not greatly different from that in the five orchards sprayed with 4–100, reduced sporodochia 98 and 92 percent respectively. Out of some 30 apricot plots (tables 4 and 5) only one instance approaching failure was noted. This occurred in Santa Cruz County in an orchard (table 5) where sporodochial development was extremely abundant and where, apparently, the spraying was not thorough.

A concentration of 1–100 (without oil) was used in four almond orchards (table 5) and one plum orchard (table 4). Efficiency in the plum orchard was probably impaired by rains occurring before the material dried on the tree. For the almond orchards located in San Joaquin County, 1–100 calcium arsenite gave variable results (table 5). In one case, improper application was suspected because of the manner in which sporodochia occurred in sprayed trees. They abounded on one side of the tree—probably the leeward during application—and were markedly less abundant on the opposite side. Similar differences were not noted in the check trees. Sporodochial suppression in the other San Joaquin County orchards was decidedly more efficient. Even 1/2–100, applied in one of these orchards, showed definite eradicant effects (orchard 16, table 5).

Effect of Added Materials on Eradicatory Properties of Calcium Arsenite

Keitt and Palmiter (*14*) tested the effect of adding various proportions of copper sulfate and lime to calcium arsenite. They concluded that injury to apple trees was reduced by large amounts of lime and that the eradicant effect against *Venturia inaequalis* in leaves was, at the same time, reduced.

In the present studies, bordeaux mixture was added to calcium arsenite primarily to reduce the arsenical injury, which was particularly severe on almonds. As arsenite injury to the trees will be discussed later, the present discussion is confined to the effect of a

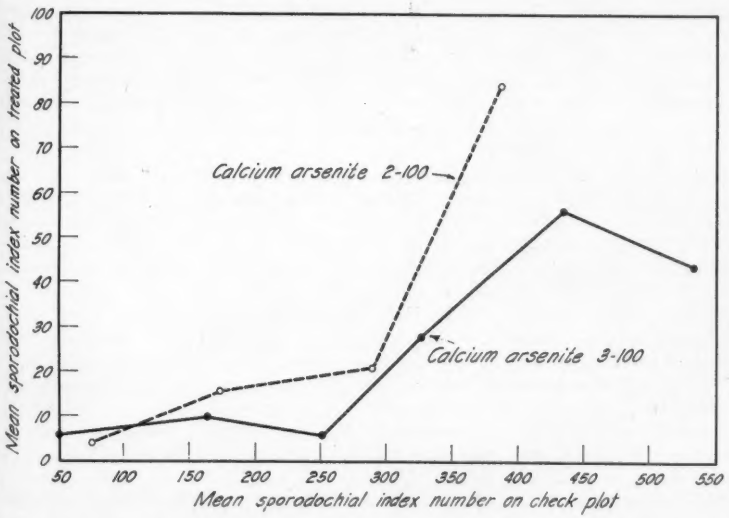

Figure 4.—Comparative effectiveness of two concentrations of calcium arsenite in suppressing sporodochial development at different levels of sporodochial abundance, 1940. The sporodochial index number was obtained by multiplying the percentage of twigs bearing sporodochia by the average number of these structures per twig. The higher the index the greater is the abundance of sporodochia.

weak bordeaux on the eradicant properties of various concentrations of the arsenite.

Bordeaux was prepared in the spray tank; and the calcium arsenite was then added. This procedure, of course, is less favorable to the formation of copper arsenite than that in which the copper sulfate and calcium arsenite are placed in the spray tank before lime is added. Though the chemistry of this combination was not studied in detail, bordeaux was found to reduce the amount of water-soluble arsenic in the spray mixture.

Turning now to the comparative effectiveness of calcium arsenite alone and in combination with bordeaux mixture, we see from table 2 that in 1939 bordeaux mixture 8–4–100 sometimes reduced the

efficiency of the arsenite. According to the summarized results in figure 3, the reduced efficiency occurred only when the concentration of calcium arsenite was 2–100 or lower. In many cases, calcium arsenite 2–100 plus bordeaux mixture 8–4–100 appeared only slightly less effective than calcium arsenite 2–100 without bordeaux. If prevention of injury to the tree by this concentration of arsenite were the primary concern, the addition of bordeaux mixture would be desirable. Other aspects of this combination will be discussed later.

In 1940 the addition of 4–2–100 bordeaux mixture to calcium arsenite 2–100 decreased somewhat the suppression of sporodochia in plum trees (table 4, orchard 5). The same combination, however, proved satisfactory in almond trees (table 5). Zinc sulfate and lime at the rate of 4 and 2 pounds, respectively, to 100 gallons of water, decreased only slightly the efficacy of 2–100 calcium arsenite (table 5). According to laboratory tests both bordeaux mixture and zinc-lime reduce the water-soluble arsenic in calcium arsenite. The inadvisability of adding concentrated bordeaux mixture is evidenced in table 5.

Petroleum-oil-emulsion in the 1938 tests (table 1) appeared to increase slightly the efficacy of calcium arsenite in preventing sporodochial development. The large amounts of oil used in these tests were not deemed necessary in later tests. In fact, 4 percent of oil appears to increase the injuriousness of calcium arsenite to apricot trees. Such is the case, for example, in the December 1938 treatments in orchard 11 (table 2). In these particular tests, neither 4 percent nor 2 percent of oil increased the eradicant properties of 4–100 calcium arsenite. According to the summarized data in figure 3, furthermore, 2 percent-oil emulsion affected 4–100 calcium arsenite little if any, and 2–100 only slightly. On the other hand, it apparently increased somewhat the efficiency of 1–100 calcium arsenite.

Petroleum-oil emulsion was added to calcium arsenite in only a few cases in 1940. It was used with 1–100 calcium arsenite in trials on almond trees (table 5), but no parallel treatments without the oil were made in the same orchard. The suppressive power of calcium arsenite 1–100 (without oil) in orchard 16 (table 5) was fully as great as that of the same concentration with oil in orchards 14 and 15. The poorer results obtained with 1–100 in orchard 17 were probably, as noted earlier, the result of improper application.

Potassium ethyl xanthate apparently had no material effect on calcium arsenite as regards either eradication of the fungus or injury to the tree (table 5, orchard 13).

Hydrolene No. 66 was added to calcium arsenite at the rate of 1 pound to 5 pounds of the latter, and the mixture was boiled in a little water for 30 minutes. After filtering and drying, the material was ground to a fine powder, which proved easily wettable and low in water-soluble arsenic. As laboratory tests showed, when a water suspension of this material was aerated for 2 or 3 days, the water-soluble arsenic rose to about the same level as that in untreated calcium arsenite. According to table 5 (orchard 14), this material used in an amount equivalent to 2–100 calcium arsenite was fully as effective as untreated calcium arsenite in suppressing sporodochia, but, as noted later, it proved no less injurious to the trees. Ap-

parently, therefore, it possesses no special qualities to warrant the extra expense of preparing it.

Effect of Applying the Sprays in December and January

Under California conditions, autumn or early winter is much more favorable for the application of sprays than midwinter or later Since spraying operations are ordinarily not delayed by heavy rains until late December, it would be advantageous to spray in autumn. The likelihood, however, that eradicant sprays would seriously injure trees in leaf prevents application until after leaf fall. Spraying cannot, in consequence, be done before the middle of November, and frequently much later.

In two apricot orchards and one almond orchard various combinations of calcium arsenite were applied a few weeks after leaf fall in December 1938; the same treatments were applied to other trees in mid-January 1939. Since few sporodochia developed on unsprayed trees until after the January applications, the effectiveness of these applications was judged on the basis of their suppression of sporodochial development.

Judging from the results in table 2, applications at both times successfully reduced sporodochial development. When, however, the results are compared in more detail, as in figure 5, certain differences are evident. Whereas December and January applications of calcium arsenite at the rate of 4–100 were equally effective, December applications of 2–100, and particularly 1–100, were less effective than January applications. In three orchards where these trials were conducted, December applications injured the tree much more than January applications. This injury to the host is taken up in a later section of the paper.

Effect of Calcium Arsenite on the Mycelium of Sclerotinia laxa in Hold-over Twigs

To obtain further information concerning the effects of calcium arsenite on *Sclerotinia laxa*, cultures were made from 25 to 30 hold-over twigs collected from sprayed and unsprayed trees 14 or more days after the sprays were applied. The surface of the twigs was treated for 2 minutes with 1–2,000 mercuric bichloride and then thoroughly washed in sterile, distilled water. The periderm of the twigs was carefully cut away with a sterile scalpel, and sections of the underlying tissue were planted on plates of potato-dextrose agar.

According to data secured during 3 years (table 6), the percentages of unsprayed twigs yielding the fungus were low in a number of cases. In fact, the number of twigs yielding the fungus in culture was lower than the number producing sporodochia. Evidently, therefore, the fungus was not obtained in culture from certain unsprayed twigs in which it was present and viable. Saprophytic fungi, present both on the surface and in the outer tissues of the twigs, appeared frequently to inhibit *Sclerotinia laxa* from developing in culture. Those present in the tissue cannot be killed by surface sterilization, and, as a consequence, sometimes overrun the plates before *S. laxa* can develop. The amount of surface sterilization possible without killing *S. laxa* in twig tissue proved to be limited, 2 to 3 minutes with 1–2,000 mercuric

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bichloride being not entirely without effect on it, and yet not always sufficient to kill all surface saprophytes.

The results of isolation studies in Table 6, however, are of value in that they support the data on sporodochial suppression given in the foregoing sections. The percentage of sprayed twigs with viable mycelium was always less than the corresponding value for unsprayed

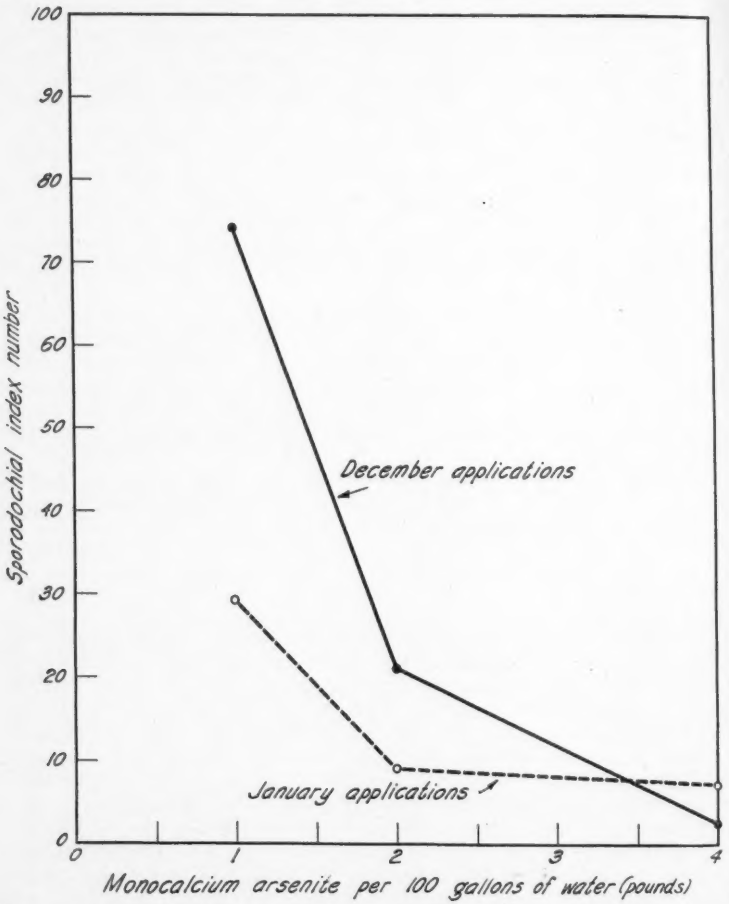

FIGURE 5.—Relation of date of application of calcium arsenite to effectiveness in suppressing sporodochial development in the winter of 1938–39. As the sporodochial index number is the product of the percentage of twigs with sporodochia and the average number of sporodochia per twig, the higher the index number the poorer is the suppression.

twigs. Thus, in three series of isolations from almond twigs in 1938, calcium arsenite 4–100 plus 4 percent oil was found to reduce the percentage of twigs with live fungus 61, 77, and 73 percent. In 1939

the reduction was as follows: Calcium arsenite 4–100 on apricot and almond twigs, 92 and 100 percent, respectively; calcium arsenite 4–100 plus 2 percent oil on apricot, a'mond, and prune twigs, 67, 91, and 90 percent, respectively; and calcium arsenite 4–100 plus bordeaux, 8–4–100, on apricot and almond twigs, 75 and 86 percent, respectively. In 1940 the percentage reductions were, for calcium arsenite 4–100 on apricot twigs, 80, and calcium arsenite 2–100 on almond twigs, 63.

TABLE 6.—*Development of mycelium of Sclerotinia laxa in blighted twigs of almond, apricot, and prune trees sprayed at different dates with two concentrations of calcium arsenite used alone and in combination with other materials, 1938–40*

Year	Kind of trees	Treatment (pounds per 100 gallons) and and date sprayed	Date of isolation	Twigs with live mycelium	Reduction due to spraying
				Percent	*Percent*
1938	Almond	Unsprayed	Mar. 2	92	
		Calcium arsenite 4–100+4 percent oil Jan. 25	do	36	61
		Unsprayed	Mar. 4	73	
		Calcium arsenite 4–100+ percent oil Jan. 25	do	17	77
		Unsprayed	Mar. 8	75	
		Calcium arsenite 4–100+4 percent oil Jan. 25	do	20	73
	Apricot	Unsprayed	Dec. 26	60	
		Calcium arsenite 4–100 Dec. 5	do	5	92
		Calcium arsenite 4–100+2 percent oil Dec. 5	do	20	67
		Calcium arsenite 4–100+bordeaux mixture 8–4–100 Dec. 5.	do	15	75
1939	Almond	Unsprayed	Dec. 27	64	
		Calcium arsenite 4–100 Dec. 13	do	0	100
		Calcium arsenite 4–100+2 percent oil Dec. 13	do	6	91
		Calcium arsenite 4–100+bordeaux mixture 8–4–100 Ded. 13.	do	9	86
	Prune	Unsprayed	Dec. 30	40	
		Calcium arsenite 4–100+2 percent oil Dec. 12	do	4	90
1940	Almond	Unsprayed	Feb. 24	51	
		Calcium arsenite 2–100 Jan. 15	do	19	63
		Calcium arsenite 2–100+Hydrolene No. 66 Jan. 15	do	17	67
	Apricot	Unsprayed	Mar. 1	69	
		Calcium arsenite 4–100 Feb. 5	do	14	80

If, for a given treatment, we compare the percentage of twigs with live mycelium with the percentage of twigs producing sporodochia, we find the latter to be the smaller. In 1938, for example, on March 8 the fungus was viable in 20 percent of the twigs sprayed with calcium arsenite 4–100 plus 4 percent of oil, whereas only 2 percent of these twigs bore sporodochia at that time. As the same trend was noted in other years, the inference is that, though the spray did not kill all of the myselium in certain twigs, it did prevent sporodochial development on these twigs. Microscopic examinations of cross sections through unsprayed twigs show the mycelium of *Sclerotinia laxa* to be most abundant in the bark tissues, but present to some extent in the wood and even the pith. Possibly, therefore, the arsenite did not kill all the myselium in the wood and pith, but did kill it in the bark tissues. As it is the mycelium in bark tissue that produces the sporodochia, killing of this mycelium probably prevents the development of sporodochia.

EFFECT OF ZINC ARSENITE ON SPORODOCHIAL DEVELOPMENT

Zinc arsenite (buffered) was applied at concentrations of 2–100 and 4–100 in seven orchards, and ½–100 and 1–100 in one orchard. As the data in table 7 indicate, its effect on sporodochial development varied considerably. In orchards 11, 12, and 9, applications of 4–100

appeared effective; but elsewhere the same strength gave poor results. Oil apparently increased the effectivene s of the 4–100 concentration in orchard 5, but was not beneficial in orchard 12. On the whole, zinc arsenite possesses much poorer eradicant properties than calcium arsenite. This conclusion grees with Keitt and Palmiter's (*14*) statement that even at 1-percent strength (8–100, approximately) the material did not always satisfactorily suppress perithecial development by *Venturia inaequalis*, whereas calcium arsenite was effective at ½ percent.

TABLE 7.—*Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with various concentrations of zinc arsenite used alone and in combination with other materials, 1938–39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index No. (A × B)	Blossoming twigs blighted
			Percent			*Percent*
Sacramento	10; almond	Unsprayed	85	6.8	578	33
		Zinc arsenite 4–100, Jan. 17	34	3.9	133	15
		Zinc arsenite 4–100+bordeaux mixture 8–4–100, Jan. 17	67	4.1	275	26
		Zinc arsenite 2–100, Jan. 17	53	3.8	201	23
		Zinc arsenite 2–100+2 percent oil, Jan. 17.	56	4.7	263	31
Contra Costa	11; apricot	Unsprayed	70	8.4	588	27
		Zinc arsenite 4–100, Dec. 5	20	2.2	44	1
		Zinc arsenite 4–100, Jan. 18	23	2.6	60	2
		Zinc arsenite 4–100+bordeaux mixture 8–4–100, Jan. 18.	55	3.1	171	3
		Zinc arsenite 2–100, Jan. 18	48	2.2	106	5
		Zinc arsenite 2–100+bordeaux mixture 8–4–100, Jan. 18.	65	5.6	364	4
Stanislaus	7; almond	Unsprayed	43	4.6	198	56
		Zinc arsenite 4–100+2 percent oil, Jan. 19.	37	1.7	63	28
		Zinc arsenite 4–100+bordeaux mixture 8–4–100, Jan. 19.	33	2.0	66	31
		Zinc arsenite 2–100, Jan. 19	45	3.0	135	42
		Zinc arsenite 2–100+bordeaux mixture 8–4–100, Jan. 19.	43	2.4	103	43
	5; apricot	Unsprayed	70	9.4	658	46
		Zinc arsenite 4–100, Jan. 20	64	5.8	371	26
		Zinc arsenite 4–100+2 percent oil, Jan. 20.	18	1.6	29	12
		Zinc arsenite 4–100+bordeaux mixture 8–4–100, Jan. 20.	75	6.0	450	.....
	6; apricot	Unsprayed	48	2.8	134	55
		Zinc arsenite 4–100, Jan. 20	38	3.4	129	18
		Zinc arsenite 4–100+2 percent oil, Jan. 20.	17	2.3	39	15
	12; apricot	Unsprayed	45	5.8	261	72
		Zinc arsenite 4–100, Jan. 19	40	1.7	68	66
		Zinc arsenite 4–100+2 percent oil, Jan. 19.	21	3.7	78	71
San Joaquin	9; apricot	Unsprayed	49	3.9	191	76
		Zinc arsenite 4–100, Jan. 19	8	2.0	16	61
	8; almond	Unsprayed	72	5.5	396	.....
		Zinc arsenite 1–100, Jan. 19	52	3.7	192	.....
		Zinc arsenite ½–100, Jan. 19	76	3.2	243	.....

Of the three arsenites used—sodium, calcium, and zinc—that of zinc is the least soluble, a fact that may partly account for its low toxicity to the fungus in the present work. Another factor might be a low sticking quality when applied to trees; but such a condition is doubtful inasmuch as this material, applied in winter, caused injury to almond trees as late as June.

Effect of Sodium Arsenite on Sporodochial Development

Information at hand showed sodium arsenite in concentrations greater than 1 pound of the salt to 100 gallons of water to be highly toxic even to apricot trees, which are more tolerant of calcium arsenite than almonds. For test purposes, however, the material was used in concentrations of 1, 2, and 3 pounds per 100 gallons of water. In one orchard 4–100 was used.

In the 1938 tests (table 1) 1.2–100 sodium arsenite was ineffective in preventing sporodochial development, but in the 1938–39 tests (table 8) it was more effective except in the earliest (December 29) application. Judging from comparative tests in orchards 3 and 4, 1–100 is much less effective than 2–100. When, however, petroleum-oil emulsion in large amounts was added to the 1–100 concentration in one of these orchards, much better suppression was effected. When this material was stronger than 1–100, injury to the tree was serious.

TABLE 8.—*Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with various concentrations of sodium arsenite used alone and in combination with other materials, 1938–39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index No. (A×B)	Blossoming twigs blighted
			Percent			*Percent*
Tehama	1; apricot	Unsprayed	49	4.9	240	39
		Sodium arsenite 1-100, Dec. 29	32	3.8	122	31
	2; apricot	Unsprayed	72	7.5	540	13
		Sodium arsenite 1-100, Feb. 8	23	1.9	44	1
Alameda	3; apricot	Unsprayed	82	7.2	590	13
		Sodium arsenite 3-100, Feb. 8	13	3.1	40	1
		Sodium arsenite 2-100, Feb. 8	19	2.4	46	2
		Sodium arsenite 1-100, Feb. 8	27	3.3	89	2
Monterey	4; apricot	Unsprayed	79	10.1	798	----------
		Sodium arsenite 3-100, Jan. 30	5	1.9	10	----------
		Sodium arsenite 2-100, Jan. 30	8	2.3	18	----------
		Sodium arsenite 1-100, Jan. 30	32	4.1	131	----------
		Sodium arsenite 1-100+5 percent oil, Jan. 30.	16	3.0	48	----------
Stanislaus	5; apricot	Unsprayed	70	9.4	658	46
		Sodium arsenite 1-100, Jan. 20	8	1.5	12	11
	6; apricot	Unsprayed	48	2.8	134	55
		Sodium arsenite 1-100, Jan. 20	9	2.3	21	8
San Joaquin	7; almond	Unsprayed	43	4.6	198	56
		Sodium arsenite 1-100, Jan. 19	20	2.2	44	30
		Sodium arsenite ½-100, Jan. 19	24	2.8	67	22
	8; almond	Unsprayed	72	5.5	396	----------
		Sodium arsenite 1-100, Jan. 18	18	2.5	45	----------
		Sodium arsenite ½-100, Jan. 18	28	3.1	87	----------
	9; apricot	Unsprayed	49	3.9	191	76
		Sodium arsenite 4-100, Jan. 19	1	1.5	2	36

Effect of Arsenites on Conidia Borne on Sporodochia Present When Sprays Are Applied

Since they occupy a relatively exposed position on the twigs, the sporodochia can be covered by sprays which would destroy either the entire sporodochium or the conidia borne thereon. Destroying the conidia without rendering the sporodochia incapable of producing new spores, or destroying the sporodochia without preventing the development of others, will, of course, cause only a temporary reduction in the abundance of inoculum. In the period of maximum development

new sporodochia may be produced within a few days. If, therefore, a spray, which destroyed these structures but did not prevent their further development, was applied, for example, 2 weeks before the trees bloomed, a new lot of spores could be produced in time to cause serious blossom infection.

Having found that calcium arsenite will prevent sporodochial development, the next step was to determine its effects on the conidia on sporodochia that were present when the spray was applied.

As noted earlier, sporodochia begin to appear on apricot and almond twigs in early January, or in some years in late December (fig. 2), and are abundant by late January or early February. As all sprays listed in the foregoing tables were applied before the sporodochia developed in large numbers, others were applied in early February to determine their effects on the conidia. At the blossoming period, or shortly after, twigs collected from sprayed and unsprayed trees were brought to the laboratory. The conidia from 25 or 30 sporodochia were washed off into separate lots of double-distilled, sterile water. By adding more water the number of spores per unit volume of each lot of suspension was adjusted to about the same value. Three drops of each suspension were placed in separate places on freshly prepared potato-dextrose agar in Petri dishes. For most reliable results—as shown by preliminary trials—observations on germination must be made after about 15 hours' incubation at 25° C. A shorter period does not allow time for all spores to germinate, and a longer period permits the germ tubes to become so long as to interfere with observations.

In addition to the germination tests, sporodochia and the conidia were examined microscopically for evidence of injury.

Almond trees sprayed on February 17, 1938, with calcium arsenite 3–100 were examined on February 25. As shown in table 9, 74 percent of the conidia from unsprayed trees germinated, whereas only 4 percent of the conidia from sprayed trees germinated.

Zinc arsenite 3–100 and zinc arsenite 3–100 plus 2 percent oil, applied to almond trees on February 23, 1938, had greatly reduced germinability of the conidia by March 1 (table 9).

The 1939 plan was to spray apricot trees in orchard 11 and almond trees in orchard 14 with all the materials used in January (table 2), but wet soil prevented this to a large extent. Certain treatments, however, were given in these orchards and in almond orchard 16 on February 9 and 15.

Material for germination tests was collected from orchard 14 on February 13, 15, and March 20, from orchard 16 on February 20 and March 20, and from orchard 11 on February 21 and March 17. As the results obtained at the different dates agreed in all details, only those of March 17 and 20 are given in table 10.

The most noticeable feature of the data in table 10 is the failure of calcium arsenite to effectively kill the conidia. A concentration of 2–100 in orchard 14 reduced germinability only 39 percent. Two percent of oil added to calcium arsenite 1–100 seemed to increase the killing power of the latter, in that conidial germinability was reduced 75 percent. The same treatment in orchard 11 reduced germinability 69 percent.

TABLE 9.—*Effect of calcium and zinc arsenite sprays on conidia borne on the sporodochia present when sprays were applied and on subsequent blossom blight in almonds, 1938*

Orchard No.	Treatment (pounds per 100 gallons) and date sprayed	Trees observed	Conidia germinating [1]	Blossoming twigs blighted
		Number	*Percent*	*Percent*
1	Unsprayed	9	74	62
	Calcium arsenite 3–100, Feb. 17	8	4	19
2	Unsprayed	5	84	24
	Zinc arsenite 3–100, Feb. 23	5	7	8
	Zinc arsenite 3–100+2 percent oil, Feb. 23	5	8	4

[1] Tests of conidial germinability were made on Feb. 25 in orchard 1 and on Mar. 1 in orchard 2.

TABLE 10.—*Effect of calcium and zinc arsenite sprays on conidia borne on the sporodochia present in almond and apricot trees when sprays were applied, and on subsequent blossom blight, 1939*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Conidia germinating [1]	Blossoming twigs blighted
			Percent	*Percent*
Sacramento	14; almond	Unsprayed	51	40
		Calcium arsenite 2–100, Feb. 9	31	24
		Calcium arsenite 2–100+bordeaux mixture 8–4–100, Feb. 9.	24	21
		Calcium arsenite 1–100, Feb. 9	20	30
		Calcium arsenite 1–100+2 percent oil, Feb. 9	13	15
		Zinc arsenite 2–100, Feb. 9	39	35
		Calcium arsenate 4–100, Feb. 9	34	34
Sacramento	16; almond	Unsprayed	66	53
		Calcium arsenite 1–½–100+ Elgetol [2] 1 percent Feb. 15.	47	42
Contra Costa	11; apricot	Unsprayed	71	36
		Calcium arsenite 1–100+2 percent oil, Feb. 15	22	8

[1] Tests of conidial germinability were made on Mar. 20 in orchards 14 and 16, and on Mar. 17 in orchard 11.
[2] Elgetol, a proprietary spray material containing 24 percent sodium dinitro-o-cresylate.

The low toxicity of these treatments is in marked contrast to the high toxicity of those given in orchard 14 (table 2) on January 17, at which time a few sporodochia were present. Germination tests on February 13 showed that the January 17 application of calcium arsenite 2–100 had reduced conidial germinability 98 percent. In fact, this treatment injured all sporodochia present at the time, many being so badly injured that the discolored, collapsed spores could be identified only by carefully separating them from the sporodochium with teasing needles. Furthermore, zinc arsenite 2–100, which in the February application (table 10) reduced conidial germinability only 24 percent, in a January 17 application (table 7) reduced germinability 65 percent.

A lack of moisture to render the arsenic soluble is believed to have been at least partly responsible for the low toxicity of the February 9 applications. Whereas rain occurred at intervals after the January 17 applications, the weather was comparatively dry throughout the last 3 weeks of February and the first week of March. Sections through sporodochia from twigs sprayed February 9 with calcium arsenite 2–100 (table 10) showed that the outermost conidia had been injured, but those in the center were normal and capable of germinating.

Keitt and Palmiter (*14*) recognized the importance of moisture to the toxic action of the copper-lime arsenites. As noted in a previous section of this paper, the addition of copper sulfate and lime (bordeaux mixture) to calcium arsenite reduced the water-soluble arsenic in the spray. Hence, even more prolonged wetting of copper-lime arsenite than of calcium arsenite would seem necessary to produce soluble arsenic in amounts sufficient to kill spores. Though no evidence on this point is at hand, the fact that zinc arsenite, the most insoluble of the arsenites used here, was less efficacious than calcium arsenite, both in preventing sporodochial development and in killing conidia, seems to support the view that rain, or moisture in other forms, is highly important to the toxicity of these materials.

In the following instance solubility seems to have been an important factor in toxicity. On February 21, 1939, applications of sodium arsenite at the rate of 1–100 and zinc arsenite at the rate of 4–100 were made to apricot trees. During the rest of February no rain fell, and there were only 2 days of rain before March 14, at which time germination tests were made. Under these conditions zinc arsenite reduced germinability of conidia only 37 percent, whereas sodium arsenite reduced it 91 percent. That is, the highly soluble sodium arsenite was the more toxic, even though at the concentrations used the total arsenic was greater in the case of the less soluble zinc arsenite.

Sodium arsenite, it will be recalled, was somewhat inconsistent in preventing sporodochial development. Possibly its high solubility accounts for this inconsistency. Heavy rains occurring shortly after application may wash it from the tree before it enters the bark and kills the mycelium of *Sclerotinia laxa*. At 3–100 and 2–100 concentrations it successfully prevented the development of sporodochia in two orchards (Nos. 3 and 4, table 8), and at a 1–100 concentration it effectively prevented their development in a number of cases where applications were made in late January or early February. The least effective application was that made December 29 in orchard 1 (table 8). Possible, therefore, weather conditions may account for the inconsistencies.

EFFECT OF A REDUCTION IN INOCULUM ON SUBSEQUENT BLOSSOM INFECTION

Because of uncertainty regarding injury by the materials, many of the tests in 1938 and 1939 and some of those in 1940 were conducted on a small number of trees. As will be noted later, the blossom infection in sprayed trees was sometimes increased by spores that drifted into them from nearby unsprayed trees. Hence, small plots were not so suitable for testing the effects of the sprays on the disease as were larger plots. This was true particularly if unsprayed trees were present to windward of small sprayed plots. In the following discussion cases will be noted where poor control was attributable to spores originating outside the treated trees.

In a strict sense, the incidence of the disease is measured by the percentage of blossoms actually invaded. The data on disease severity obtained in these studies were not based on the percentage of blossoms attacked for the following reasons. Upon entering a blossom the fungus may quickly grow into the spur or terminal on which the blossom is borne. Shortly after the invaded blossom shows the first signs of the disease, other blossoms on the twig, such as those in the same

cluster, and those distal to the infected blossom, die because the twig is girdled. Unless observed just before they die the blossoms killed by girdling are difficult to distinguish from those invaded by the fungus. When tests are extensive and are located in widely separated districts where blossom infection occurs at different times, each orchard cannot be examined at the proper time. In the present studies, therefore, observations were delayed until blighting of twigs was accomplished, thus allowing the symptoms to reach the same stage in all orchards regardless of when infection occurred.

Each blossom-bearing spur and terminal was considered a unit and the percentage of these units blighted was determined by counting 200 at random in each tree. An abnormal blossom development in 1940 necessitated a second method for taking records in certain districts. Blighted spurs and terminals were counted on five selected branches in each tree.

These methods of recording disease occurrence, of course, do not yield strictly quantitative data. The sporodochial index numbers, it will be recalled, measure the frequency of sporodochia (and consequently conidia) on the hold-over twigs, but do not indicate the total number in a tree. In view of these facts we must not expect strictly proportional relations between the two sets of data. On the whole, the disease index number will be high when the sporodochial index number is high, and low when it is low, but to obtain similar control in two given orchards where the number of hold-over twigs differs greatly, the efficiency of sporodochial supression must be higher in the orchard with the greater number of hold-over twigs. Hence, we may expect to find cases where the same degree of sporodochial supression results in different control.

The relations between primary inoculum level and disease level may possibly be disturbed by still another factor. As infected blossoms produce conidia soon after they wilt, these conidia might be responsible for further blossom infection. If such is the case, a few infected early blossoms may produce sufficient conidia to cause a severe outbreak of the disease in blossoms which open later, even though the eradicant spray had efficiently suppressed the primary inoculum. Though secondary infection of this sort is probably of minor importance in a warm spring, it might be important in years when low temperatures or other factors prolong the blossoming period. Except under some such conditions the period of blossom susceptibility, extending from emergence of the blossoms to petal fall, is so short that the earliest infected blossoms scarcely have time to produce conidia before the petals are off all blossoms.

Blossom Blight in Trees Sprayed Before Many Sporodochia had Developed

In 1938 significant reduction in severity of blossom infection (table 1) accompanied the application of calcium arsenite before sporodochia appeared. Less important reduction followed applications of zinc and sodium arsenite. The relation between sporodochial abundance and disease severity is seen when the data in table 1 are represented as percentage sporodochial prevention and percentage disease reduction. Thus, calcium arsenite 4–100 reduced sporodochia 95 percent and the disease 77 percent; calcium arsenite 4–100 plus 4 percent of oil, 99

and 79 percent, respectively; zinc arsenite 4–100, 88 and 52 percent; zinc arsenite 4–100 plus 4 percent of oil, 80 and 57 percent; and sodium arsenite 1.2–100, 33 and 16 percent.

The 1939 trials were conducted under a variety of climatic conditions and in orchards where the past history of the disease varied considerably, and in which, as we saw earlier, production of inoculum by the fungus differed greatly. Hence, we may expect variabilities in the results. According to data in tables 2, 3, 7, and 8, it may be concluded that blossom blight decreased markedly with high degrees of sporodochial suppression, although not invariably. In other cases, furthermore, an equally low incidence of the disease occurred in plots where the degree of sporodochial suppression differed greatly. The cause of these exceptional cases must be sought among factors such as the abundance of hold-over twigs, the presence of wind-borne conidia, and others.

Both calcium and zinc arsenites, for example, gave satisfactory disease control in orchard 11 (tables 2 and 7), though sporodochial suppression by the latter was only a fraction of that by the former. Granting that our knowledge of the factors responsible for these differences in results is not complete, nevertheless several of the more important are known. Where the index numbers show a low degree of sporodochial suppression, either because the material was ineffective or because it had not been applied properly, we should expect little reduction in the disease. Aside from the exceptional control of blossom infection in orchard 11 (table 2), the data conform to this view. Accordingly, it must be concluded that certain differences existed between orchard 11 and other orchards. As noted earlier, the total number of hold-over twigs per tree contributes to the severity of blossom infection: the greater the total number of spores, the greater the chances for infection. Differences in the total hold-over sources arise from differences in severity of the disease the previous year, or from differences in the size of trees, or both. The trees in orchard 11, for example, were smaller than those in many of the other orchards; and, though the incidence of disease had been high in past years, the total number of hold-over twigs per tree was low as compared with that in other orchards. Orchard 11A, located 1 mile from orchard 11, represented a different set of circumstances; the disease was severe in previous years, the trees were large, and, in consequence, the total number of hold-over twigs was greater in these trees than in those of orchard 11. Although in untreated plots of orchard 11 the level of sporodochial development exceeded that in untreated plots of 11A, the incidence of disease was higher in the latter orchard. Parallel treatments of calcium arsenite 4–100 reduced sporodochial abundance 99.5 percent in orchard 11 and 99 percent in orchard 11A; yet the disease was less effectively controlled in the latter than in the former. In other words, the disease was more difficult to control in orchard 11A because it was necessary to obtain a higher relative degree of sporodochial suppression in order to overcome the difference in total sporodochial numbers.

The results secured in orchard 11A, therefore, represent more rigid tests of the efficiency of calcium arsenite. This conclusion does not, of course, invalidate the results secured in orchard 11, for here the significance of disease reduction was attested by the abundance of

blossom blight developing in three unsprayed plots located in different parts of the orchard.

Unsatisfactory control of blossom infection followed application of calcium arsenite in orchards 12 and 9 (table 3) even though suppression of the fungus was highly effective. The plots in these orchards as well as in orchard 5 (table 3) were small and, in consequence, were near untreated trees bearing large numbers of sporodochia. In orchard 9, for example, the plot receiving calcium arsenite 4–100 on November 30 consisted of six trees, two adjacent to the untreated block, and the rest not more than two or three rows from other untreated trees. In orchard 12, treated blocks two rows wide and four trees long were so situated that untreated trees bordered them both on the north and on the south. The treated trees in both orchards were therefore exposed to wind-borne spores from other trees. Evidence that spores from sources outside the treated trees caused infection was present in orchard 9; the two treated trees adjacent to the control trees were more severely infected than the other trees receiving the same treatment.

Additional evidence that spores drift from one tree to another was secured in Contra Costa and Tehama Counties. In two orchards untreated trees were located south of plots receiving calcium arsenite. In both cases the treated trees adjacent to the untreated plot developed blossom blight more abundantly than trees three or four rows away. The effect of drifted spores was also noticeable in the second treated row. During periods of rain at blossoming time, southwest winds with velocities of 14 to 16 miles per hour occurred in these districts. In small plots or in plots located leeward of untreated trees bearing sporodochia it seems not unlikely, therefore, that wind-borne conidia or conidia in wind-borne rain may vitiate the disease control otherwise obtainable with eradicant sprays.

Data in table 2 reveal that the addition of bordeaux 8–4–100 and oil emulsion (2 percent) had no marked effect on disease control by calcium arsenite 4–100 in orchard 11A. Bordeaux, however, somewhat reduced the efficacy of calcium arsenite 4–100 in orchard 14 (table 2) and markedly reduced the efficacy of December applications of 2–100 and 1–100 concentrations.

Calcium arsenite 2–100, alone and with oil, gave excellent control in several instances. Aside from orchard 11 (table 2), where control was good and was easily accomplished, this concentration reduced the disease as follows: In orchard 14 (table 2) December applications, 2–100, 88 percent, and 2–100 plus 2 percent of oil, 79 percent; January applications, 2–100, 82 percent, and 2–100 plus 2 percent of oil, 88 percent. In orchard 5 (table 3), 2–100 plus 2 percent of oil reduced the disease 65 percent.

A December application of calcium arsenite 1–100 in orchard 14 (table 2) reduced the disease 73 percent. December and January applications of calcium arsenite 1–100 plus 2 percent of oil were accompanied by reductions of 70 and 67 percent, respectively. Evidently, therefore, rather dilute suspensions of this material gave significant control.

Except in orchard 11, zinc arsenite was comparatively ineffective against blossom blight. The results in table 7 indicate some control at a 4–100 concentration, but no case equaled the best obtained with calcium arsenite.

Except for orchard 9 (table 8), where the amount of disease in treated plots was apparently increased by spores drifting in from untreated trees, the control with sodium arsenite paralleled, on the whole, the eradicant efficiency. In orchard 1, sodium arsenite 1–100 effected only a moderate reduction in the number of sporodochia, and practically no reduction in blossom infection. In orchards 2, 3, 5, and 6, on the other hand, both sporodochial numbers and incidence of the disease were reduced greatly. In orchard 7, control was noticeable but not satisfactory. The inconsistent results with sodium arsenite may be explained, in part, by its high solubility. It apparently has considerable immediate effect on sporodochia present when it is applied, but seems to lose this effect during heavy rains. The composition of sodium arsenite solutions used in these tests probably varied, and this variation may have caused some of the inconsistencies.

As the disease was absent in certain districts in 1940, no data were obtained. Results, however, were obtained in 12 orchards, blossom infection being very abundant in 5 and moderately abundant in 7. Because of abnormal blossom development in some districts two methods were employed in collecting the results. In orchards where the trees blossomed normally, the percentage of blossoming shoots blighted was determined in the same manner as in earlier years (table 4, orchards 1 to 5). In orchards where blossom buds developed abnormally, the number of blighted blossoming twigs was counted on selected branches (table 4, orchards 6 to 12).

Except in orchard 9 (table 4) and in the case of calcium arsenite, 1–100, in orchard 5 (table 4), satisfactory reduction in blossom infection resulted from suppressing the sporodochia. The occurrence of abundant blossom infection in orchard 9 despite rather effective prevention of sporodochial development is believed to have resulted from wind-borne conidia from nearby unsprayed trees. This plot was small and was located leeward of unsprayed trees. In case of the calcium arsenite 1–00, sporodochial suppression was not highly effective, possibly because rains occurred before the spray had thoroughly dried.

In Contra Costa and Tehama Counties where the disease was severe, blossom blight was reduced 85 percent in orchard 1, 97 and 95 percent in orchard 2, 98 percent in orchard 3 (table 4), 91 percent in orchard 6, and 78 percent in orchard 7 (table 4). Through the data are not numerous enough to allow comparisons of various concentrations, they suggest that both 3–100 and 2–100 were highly effective under the 1940 conditions.

Opportunity was offered in 1940 for comparing the control given by calcium arsenite alone, calcium arsenite followed by the standard applications of bordeaux in the preblossom stage, and bordeaux in the preblossom stage. The spring of 1940 proved unfavorable for spraying, in that rains occurred frequently during the preblossom stage. A relatively short dry period just as the blossoms emerged from the bud was sufficient to allow certain orchardists to apply bordeaux mixture. The rains responsible for the major part of blossom infection occurred within a few days after application of the spray. In most of these cases bordeaux effectively controlled the disease.

According to data in figure 6, the eradicant sprays, calcium arsenite 2–100, 2½–100, and 3–100, compared favorably with bordeaux 10–10–100, 12–12–100, and 16–16–100 with respect to reduction of

blossom infection. In three out of four cases, a calcium arsenite spray followed by a bordeaux spray effected moderately better control than when calcium arsenite alone was applied; and in one of the four cases, control was significantly better. Neither bordeaux alone, calcium arsenite alone, nor calcium arsenite followed by bordeaux gave satisfactory control in one of the six orchards. In this orchard the calcium arsenite plots were small and, in consequence, exposed to wind-borne conidia from unsprayed trees.

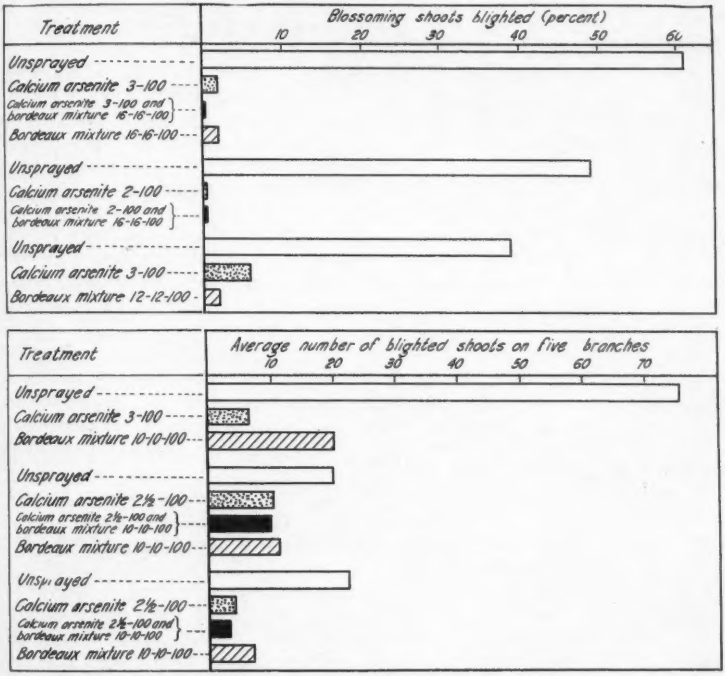

FIGURE 6.—Control of apricot blossom blight by winter applications of calcium arsenite and by preblossom applications of bordeaux mixture, 1940. Quantities given in pounds per 100 gallons.

BLOSSOM BLIGHT IN TREES SPRAYED AFTER SPORODOCHIA HAD DEVELOPED IN LARGE NUMBERS

Tables 9 and 10 contain results of the few treatments given when sporodochia were present in the trees in large numbers. In 1938 the material reductions in conidial germinability attending applications of both calcium arsenite 3–100 and zinc arsenite 3–100 were accompanied by a fairly satisfactory reduction in blossom infection. In 1939, on the other hand (table 10), inasmuch as the sprays proved ineffective in killing conidia, the disease was not reduced greatly. An exception was the 78 percent reduction of the disease attending application of calcium arsenite 1–100 plus 2 percent of oil in orchard 11. This orchard, it will be recalled, proved exceptional in cases of all other applications.

INJURIOUS EFFECTS OF THE ARSENITES

Injury to the tree was soon found to limit somewhat the usefulness of arsenites as eradicant sprays. Sodium arsenite, probably because of its high solubility, was more injurious than calcium or zinc arsenite. For this reason, concentrations above 1 pound of the salt per 100 gallons of water were used in only a few instances. Apricots appeared able to tolerate 1–100 sodium arsenite, provided the trees were dormant when sprayed. Almonds proved so highly sensitive to all the arsenites that no concentration effective against the fungus could be used with safety. The varieties of plums and prunes tested appear tolerant towards effective concentrations of calcium arsenite.

Injury was manifested in several ways. Any comparatively fresh wound permits the absorption of soluble arsenic with resultant killing of tissue around the point of entry. Pruning wounds, therefore, were common points through which arsenic gained access to bark tissue. Upon entering the cut ends of branches the arsenic would sometimes kill the bark tissue for a considerable distance from the end. Lateral twigs arising from these killed areas were girdled and killed. When pruning operations were delayed until after application of the spray, much of this injury was avoided, although in certain instances soluble arsenic entered wounds made after the trees were sprayed. Such occurrences, however, did not ordinarily cause material damage.

Arsenic entered directly through the unbroken bark to some extent in almonds, but usually not in apricots. Judging from the effect of arsenites on mycelium in hold-over twigs, one would expect the bark of dead twigs to be highly permeable to soluble arsenic. Some minor injury occurred in almonds, where the arsenic, upon entering dead twigs, was carried downward into the live portions. Such injury was not observed in apricots or plums.

A problem with almond trees was the killing of young shoot growth in early summer by arsenites applied the previous winter. After the first period of high temperatures in late May or early June, clusters of leaves on terminals and spurs suddenly wilted, the cause being the death of tissue at the bases of currently developing twigs. Since the sprays were applied before these twigs developed, injury probably arose from soluble arsenic washed over these tissues by rain. In all cases, light showers preceded the injury by a week or more. Neither apricots nor plums developed this type of injury.

Because arsenites are extremely toxic to plant tissue unprotected by a thick periderm, the eradicant sprays must be applied only while the trees are dormant; that is, between leaf-fall in the autumn and the swelling of buds in the spring. Since autumn weather conditions are favorable for spraying, special advantages would arise from treatment at this time. With this fact in mind, sprays of various concentrations and combinations of calcium arsenite were applied in December 1938 and January 1939. The December sprays were delayed until 2 to 3 weeks after leaf-fall. The relation of the time of application to sporodochial suppression was discussed earlier. In all three orchards—two of apricot and one of almond—December applications produced far more injury than January applications. For example, 4-100 calcium arsenite applied in December killed twigs of ½ to ¾ inch diameter, whereas the same concentration applied in January killed only a few of the smallest twigs. Bud injury, with

consequent reduction of crop, was especially noticeable on trees sprayed in December, but absent from those sprayed in January. The difference was found to be connected with the leaf-scars. For a time after leaf-fall the vascular elements of leaf-scars are apparently accessible to soluble arsenic, which enters and kills the tissues at the base of buds arising near the scars. After December spraying, enough arsenic was absorved through leaf-scars to kill large twigs as well as individual buds on these twigs. By January, however, the leaf-scars were apparently no longer permeable to the arsenic, and bud and twig injury was not produced even by 4-100 calcium arsenite, except where the arsenite entered pruning wounds.

The following observations pertain to the effects of supplementary materials on injuriousness of December applications of calcium arsenite. Two-percent oil emulsion increased injury slightly, whereas 4 percent increased it materially (table 2). Bordeaux mixture 8-4-100 reduced injury of 4-100 calcium arsenite, but not to the point where this combination was safe. Calcium arsenite 2-100 plus bordeaux 8-4-100 produced little injury as compared with calcium arsenite 2-100. Except calcium arsenite 1-100 and its combinations, however, all December applications were far more injurious than January applications.

Zinc arsenite treatments paralleling these calcium arsenite treatments proved much less injurious, the only noticeable damage occurring on almond trees sprayed in December. Since, however, this material possesses low eradicatory properties, there is no reason for preferring it to calcium arsenite.

In the extensive trials of 1940, calcium arsenite caused material injury in only 3 out of about 30 apricot orchards. In one of these 3 cases 4-100 calcium arsenite was applied to trees injured by sodium arsenite the previous season. Four other orchards in the same district sprayed with this concentration developed no injury. In another orchard petroleum-oil emulsion was added to 3-100 calcium arsenite. Branch injury was rather severe. The third case of injury occurred in a Contra Costa orchard sprayed on February 9 with 2-100 calcium arsenite (table 4, orchard 3). At this date the blossom buds were swelling, and arsenic entered and damaged them somewhat. The spray was applied with a large rig operating at about 500 pounds' pressure, which is believed to have driven the spray into the buds, since no injury followed the spraying at 250 pounds' pressure on February 9 in Solano County (table 5).

The primary purpose of the 1940 tests in almond orchards was to determine the injurious effects of calcium arsenite 2–100 and 1–100 alone and combined with other materials (table 5). The following information was obtained: (1) Conditions in 1940 were much more conducive to injury than in 1939. The amount of rainfall following the application was much greater in 1940 than in 1939. (2) Though both zinc-lime 4–2–100 and bordeaux mixture 4–2–100 reduced the injury caused by 2–100 calcium arsenite in the winter, neither noticeably reduced the injury caused in early summer. (3) Potassium ethyl xanthate proved ineffective in reducing injury. (4) Although Hydrolene No. 66, at the rate of 1 pound to 5 pounds of calcium arsenite, temporarily lowered water-soluble arsenic, it did not reduce injury. (5) Even at concentrations of 1–100 the calcium arsenite did consid-

erable damage that appeared in the summer as a withering of leaf clusters.

To determine the effect of bordeaux applied in the preblossom stage on the injury which develops in summer, two blocks of almonds previously sprayed with calcium arsenite 2–100 (table 5, orchards 14 and 15) were sprayed with a 12–12–100 concentration of the material. Injury was reduced but not avoided. Such prevention of injury as did occur was probably the result of the fact that the excess lime in the bordeaux mixture rendered the calcium arsenite less soluble for a time. In an earlier discussion bordeaux was said to reduce the solubility of calcium arsenite in laboratory tests. In these tests lower solubility of the arsenite also followed the addition of lime, but upon aerating a suspension of calcium arsenite and lime for a few days the water-soluble arsenic rose to about the same level as that in a suspension of calcium arsenite without lime. On the other hand, bordeaux added to calcium arsenite not only reduced the water-soluble arsenic about as effectively as lime, but it also prevented a material increase in water-soluble arsenic during aeration of the suspension.

SUMMARY AND CONCLUSIONS

A serious blossom blight caused by *Sclerotinia laxa* is common on several stone-fruit species in California. Apricots and almonds in particular are liable to attack by the fungus, which upon entering the blossoms proceeds to invade the twigs, thus destroying much of the fruiting wood. Although this fungus sometimes attacks ripening fruit, most fruit rotting is attributable to *S. fructicola*.

As no apothecial stage of *Sclerotinia laxa* has been found in California, the entire cycle is believed to be confined to the tree. Conidia produced on blighted blossoms during the summer probably do not survive the winter. Instead, the mycelium in the blighted twigs, the invaded blossoms, and the occasional mummied fruits that hang in the tree will give rise to sporodochia during the winter. To judge from observations during three seasons, sporodochial development begins sometime in midwinter and continues for a month or more. The conidia produced on these sporodochia are the primary inoculum for blossom infection in the spring.

The present program for controlling blossom blight consists in spraying the trees with a protective spray, such as bordeaux mixture, when the blossoms emerge in the spring, and in removing the blighted hold-over twigs and fruit. Certain difficulties attend this program.

The presence in the tree of the only known hold-over stage of *Sclerotinia laxa* affords opportunities during the dormant season to reduce the survival level by spraying with eradicant fungicides. Depending upon the time of application with respect to sporodochial initiation, the eradicant fungicide would either suppress the development of these structures, or destroy them after they developed.

The experiments herein reported covered a 3-year period and consisted of approximately 161 trials of monocalcium, zinc, and sodium arsenites in apricot, almond, prune, and plum orchards located in 15 counties. The size of plots ranged from 4 trees to as high as 120. The records secured dealt with the effect of the arsenites (1) on sporodochial development, (2) on conidia present on the sporodochia when

sprays were applied, and (3) on the mycelium of the fungus in blighted hold-over twigs.

Although possessing eradicatory properties, both zinc and sodium arsenites were found to be inferior to calcium arsenite. Sodium arsenite, furthermore, proved too injurious when used in its most effective concentrations.

In many of the tests calcium arsenite, 4 pounds per 100 gallons of water, reduced sporodochial development 98 to 99 percent. Lower concentrations (3–100 and 2–100) proved somewhat less effective, but even with a concentration as low as 1–100 the eradicatory effects were noticeable. A concentration of 4–100 proved too injurious even to apricot trees, which are much more tolerant of arsenites than almonds. The concentration that appears comparatively safe to use on apricots and which possesses a high eradicant efficiency is 2–100, or possibly 2½–100.

Various materials were added to calcium arsenite in a study of their effects upon its eradicant and injurious properties. Of the materials used, petroleum-oil-emulsion alone increased eradicant properties, particularly of 2–100 and 1–100 calcium arsenite. Oil in large amounts (4 percent) tended to increase injury to apricot trees. At concentrations of 8–4–100 or even 4–2–100, bordeaux mixture and zinc-lime decreased both the eradicant and the injurious properties of the lower concentrations of calcium arsenite, but affected slightly the higher concentration (4–100). Materials such as potassium ethyl xanthate and Hydrolene No. 66 added nothing to the efficacy of calcium arsenite.

Although further tests might show that variations in the copper, lime, and arsenite components of the calcium arsenite-bordeaux mixture combination would produce a spray combining greatest safety with highest efficacy, the results thus far indicate that the safety feature can be enhanced only at the expense of eradicant efficiency. According to laboratory tests, bordeaux mixture added to calcium arsenite lowered the water-soluble arsenic content of the spray. The same was accomplished by adding lime; but, whereas lime retained soluble arsenic at a low level only so long as it remained unaerated, bordeaux continued to suppress soluble arsenic even after days of aeration.

Cultures made from blighted twigs sprayed with calcium arsenite indicate that some material entered the twigs and killed the mycelium of *Sclerotinia laxa*, thus preventing the production of sporodochia. This material is probably water-soluble arsenic, inasmuch as materials, such as bordeaux, which reduced water-soluble arsenic also reduced the eradicant effects of the weaker calcium arsenite concentrations.

Arsenites applied to the trees after sporodochia developed in large numbers were less consistent in destroying the fungus than were those applied before sporodochia developed. In 1938 both calcium and zinc arsenite killed a high percentage of conidia borne on sporodochia present when sprays were applied in February. In 1939, whereas January sprays effectively destroyed conidia then present, February sprays injured the outermost spores on the conidiophores but failed to affect those on the interior of the tightly packed mass of conidial chains. Lack of sufficient rainfall—only 0.1 inch occurred between

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applications and blossoming time—to liberate soluble arsenic is believed responsible for this low toxicity.

Abundant blossom infection in the experimental orchards afforded opportunities to observe the relations between reduced sporodochial abundance (hence conidial abundance) and subsequent incidence of disease. On the whole, efficient suppression of sporodochial development brought marked disease reduction. In a few cases where treated plots were small, and hence located near untreated trees, control of the disease was not commensurate with sporodochial suppression. There is evidence that much of the infection in these plots came from conidia produced outside the treated plots and brought in by wind or by wind-borne rain.

Examples of the reduction in blossom infection obtained with calcium arsenite are as follows: 1938, 70 and 77 percent; 1939, 91 and 96 percent; 1940, 85, 91, 97, and 98 percent. The degree of disease reduction following applications of zinc and sodium arsenite paralleled their eradicant efficiencies, which, as noted earlier, were subject to fluctuations.

Control of blossom blight in plots receiving only calcium arsenite compared favorably with that in plots receiving bordeaux mixture in spring, and with that in plots receiving both the calcium arsenite and the bordeaux treatments. The eradicant treatment, however, is not expected to replace the bordeaux treatment, but to supplement it.

The injurious effects of arsenites on the tree are reported. Injury may develop from entry of arsenic into wounds, leaf scars, buds, dead twigs, or—in the almond—directly through uninjured bark. Almond trees proved so sensitive to arsenites that no effective concentration could be used on them with safety. Apricots and the plum varieties tested were more tolerant. Apricots were injured seriously only when high concentrations of arsenites were used, when applications were made soon after the trees were pruned, or when the trees were sprayed soon after the leaves fell in the autumn. Concentrations of 2–100 or 3–100 seem safe if applied in midwinter. To delay pruning until after the spray is applied avoids much of the injury through wounds. Injury through leaf scars can be minimized by postponing spraying until midwinter, at which time the leaf scars appear no longer to afford a passageway for water-soluble arsenic to enter twigs.

Although bordeaux mixture in weak concentrations (4–2–100 and 8–4–100) reduced injury to some extent, it also decreased the eradicant effect of the arsenite. Oil in large quantities increased injury, while potassium ethyl xanthate and Hydrolene No. 66 produced no change in this respect.

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A COMPARISON OF METHODS OF QUADRATTING SHORT-GRASS VEGETATION [1]

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INTRODUCTION

Permanent quadrats have long been employed in range studies where it is desired to measure the progress of changes in vegetation as a result of different systems of grazing. In some studies, description of the vegetation on such quadrats is effected by means of charts showing the position and extent of each plant; in other cases, estimates of the basal area of the vegetation or of its crown spread are made; in others the total weight or volume of each species is estimated; and in still others the plants are simply counted. The method to be used is determined largely by the characteristics of the vegetation; the method of counting would not be feasible with turf-forming species such as the short grasses, for example, but it might be useful in associations of single-stemmed plants.

In short-grass associations, descriptions of quadrats tend to be expressed in terms of area of ground cover, because of the low, matted habit of the principal species. There are many ways of determining area of ground cover—the first and second methods mentioned in the last paragraph are two of these. Until recently no critical comparisons have been made between methods. The present paper reports the results of a comparison made near Miles City, Mont., with the specific objectives of determining the degree of consistency of three methods and the causes of subjective error in each.

To the writer's knowledge, the only published comparisons of methods made prior to the present one were by Hanson and Love (*5*) [2] in Colorado, and by Hanson (*4*) in North Dakota. These studies emphasize the limitations of methods as affected by the growth habits of different prairie species, but do not treat of errors of estimate. More recently West (*15*) has reported on the degree of correlation between repeated estimates of the same vegetation by the density-list method as used in the Union of South Africa.

[1] Received for publication September 4, 1941. The field work of this study was carried out at the U. S. Range Livestock Experiment Station, Miles City, Mont., where the Northern Rocky Mountain Forest and Range Experiment Station of the Forest Service conducts range investigations in cooperation with the Bureau of Animal Industry and the Montana Agricultural Experiment Station. Part of the material contained in this paper was presented to the faculty of the Graduate School of the University of Minnesota as a thesis in partial fulfillment of the requirements for the degree of master of science.

[2] Italic numbers in parentheses refer to Literature Cited, p. 614.

EXPERIMENTAL PROCEDURE

METHODS TESTED

Three methods—pantograph-chart, density-list,[3] and point-analysis—were tested. For convenience the three names are shortened in the body of this paper to "chart" for pantograph-chart, "list" for density list, and "point" for point-analysis.

The chart method, illustrated in figure 1, has been described by Hill (*6*) and McGinnies (*9*). It consists essentially of reproducing a plan view of the vegetation on cross-section paper, from which areas of the various tufts can be determined later in the office.[4]

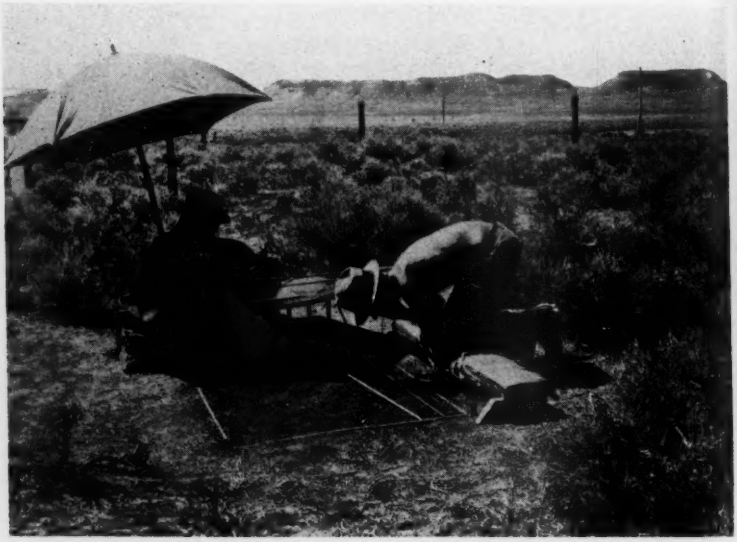

FIGURE 1.—In charting with the pantograph, the observer (right) outlines vegetation with the pointer, and the tracing is duplicated in reduced scale on the chart.

By the list method, an improved form of which has been described by Murray and Glover (*10*), the quadrat is divided into square decimeters, and with the aid of a small sliding frame carrying cross wires (fig. 2), the number of square centimeters of vegetation per square decimeter is estimated.

[3] The term "density-list" is not a particularly happy one, since areas in square centimeters, not densities in percent, are listed. The apter term "area-list" has already been used (*5*) to denote a method by which areas of separate tufts are recorded; therefore "density-list" is adopted for the present comparison. With a listing unit of 100 cm.^{2}, as used in this comparison, the two terms come to the same thing.

[4] Records by the chart method differ from those obtained by other methods in that single shoots or very small tufts are marked by spot symbols and stolons by lines, whereas by the list and point methods such small bits of vegetation are converted directly into terms of area. In order to be able to make such a conversion from the quadrat charts, each observer was asked to submit his independent estimate of the number of spots and length of stolon which he considered equivalent to a square centimeter. The estimates were closely similar, and they have been averaged in reducing the data on the charts to an areal basis. Because the outlining of very small areas by pantograph is time consuming and seldom successful, on account of play in the instrument, tufts which in the observer's judgment were between 0.5 and 1.5 cm.^{2} in area were drawn in by the recorder as areas of 1 cm.^{2} These conventions probably tend to reduce somewhat the variation between observers using the chart method. In order to minimize mechanical errors the same pantograph was used for all trials on any one quadrat. Two were used, both of the type shown in figure 1, described by Pearse et al. (*11*).

The method of point-analysis [5] which has been employed by New Zealand workers since 1925 (*2*, *7*, *8*), and was here adapted for use on quadrats, is a record of the proportion of evenly spaced, vertically projected pin points which strike vegetation. Thus, if 100 such points are projected downward over an area of 400 cm.^{2}, and 10 strike grass while 90 strike bare ground, the estimate will be that the density of the grass is 10 percent or that 40 cm.^{2} of the 400 is grass. The essential apparatus is shown in figure 3. A series of holes in the horizontal rack enables the observer to look down along each pin, materially increasing his certainty whether a hit is made, and revealing at a glance whether a hit is impossible.

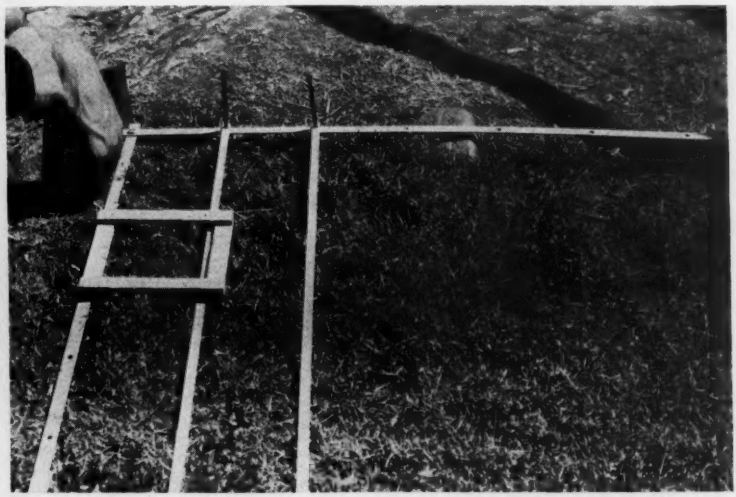

FIGURE 2.—The sliding density-list frame encloses a square decimeter divided into 25 equal parts by cross wires. Within it an estimate is made of the number of square centimeters filled with vegetation. In practice the straps dividing the quadrat into decimeter strips are offset to compensate for their width.

The point method gives an estimate of the amount of vegetation by a sampling process; consequently a portion of the observed variation may be attributed to sampling errors as well as to variations in judgment between observers. Variations in results by either chart or list method, if the area estimated be constant, may be attributed almost wholly to variations in judgment.

SELECTION OF QUADRATS

Three quadrats of varying density were selected for the test (fig. 4). Quadrat 1 (1 by 0.5 m.) had a sparse cover of blue grama grass (*Bouteloua gracilis*) in small, somewhat scattered, and fairly well-defined tufts. Quadrat 2 (0.5 by 0.5 m.) supported a denser stand of mixed

[5] The point-observation-plot method, a term introduced in 1936 by Stewart and Hutchings (*14*) to describe a large-scale estimate method, has no connection with this.

grama and buffalograss (*Buchloë dactyloides*) in larger, less well-defined tufts. Quadrat 3 (0.5 by 0.5 m.) supported a high density of buffalograss forming a matted turf with no clear distinction as to tufts.

Other species on the quadrats were negligible and are omitted from the computations that follow. The two major species are so similar in growth form that they have been regarded as one in this purely quantitative test. Tufts of buffalograss lacking stolons and inflorescences are frequently confused with grama, even by trained field men.

Quadrats smaller than the customary square meter were selected because experience has shown that if 3 meter-square quadrats were to

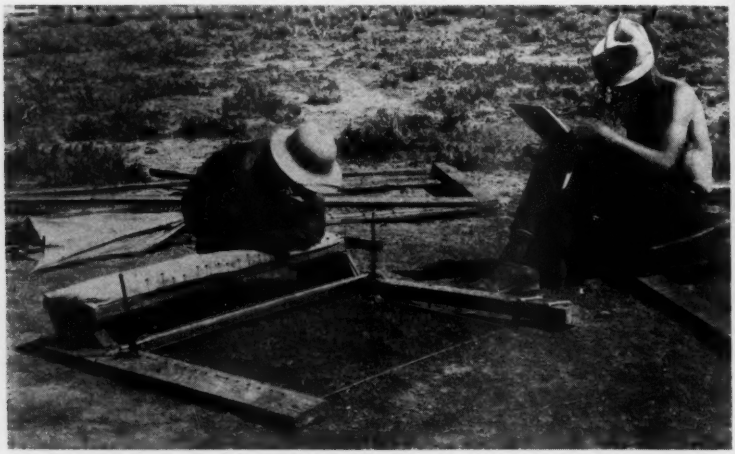

FIGURE 3.—With the point analyzer set up at the rate of 400 points per square meter the observer (left) pushes sharpened pins down onto the quadrat one by one, and calls the hits. Then the rack is moved to the next pair of holes in the baseboards and the process is repeated.

be estimated 4 times by 5 men using 3 methods—a total of 180 estimates—the area of grass might undergo appreciable change during the time required. More rapid estimates could be made on smaller quadrats. The question naturally arises: Can estimates on a smaller quadrat be considered comparable to estimates on an entire square meter? A partial answer was found in the charts of a meter-square quadrat, made the previous year by 5 men. On this quadrat of mixed grama and buffalograss the observers' estimates varied from 2,229 to 4,678 cm.^{2} Breaking each chart into quarters and expressing the area of vegetation on each quarter in percent, it is evident, as shown by table 1, that although the estimates varied widely, the observers were fairly consistent from quarter to quarter. There is reason to suppose, therefore, that if only a quarter of the square meter had been charted the relative differences between men would have been similar to those observed on the entire quadrat.

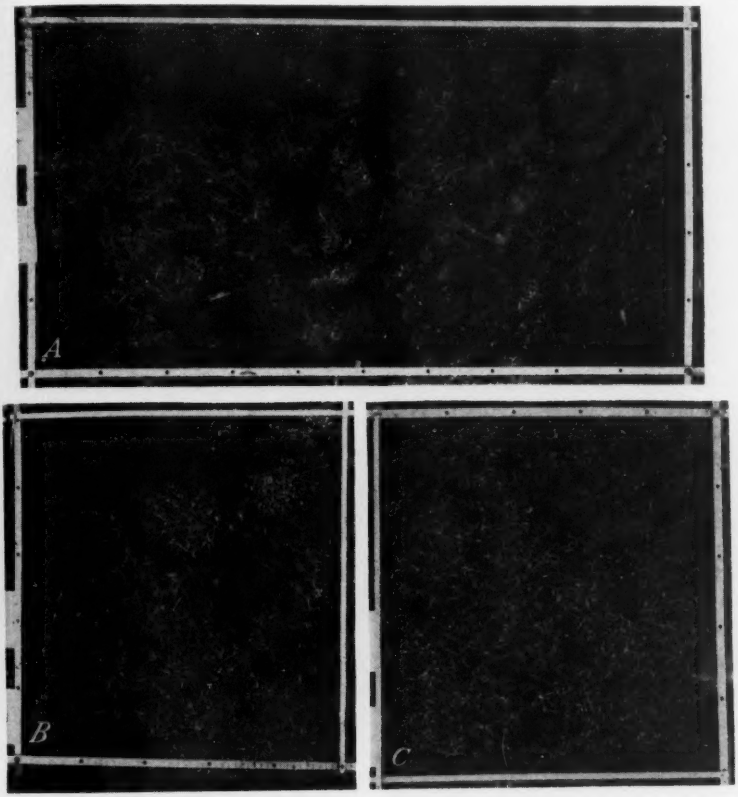

FIGURE 4.—The three quadrats used in the test. *A*, Quadrat 1, double the size of the others, mainly blue grama. *B*, Quadrat 2, predominantly buffalograss, with some grama. The prominent tuft 12 cm. from north edge, center, is grama; buffalograss, such as the tuft to the right of this, can be distinguished by stolons. *C*, Quadrat 3, practically pure buffalograss.

TABLE 1.—*Comparison of independent observers' estimates by quarter quadrats, in percent of total estimated*

Observer	Northwest	Northeast	Southwest	Southeast	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
I	26.2	23.1	24.2	26.5	100.0
J	26.0	23.8	24.6	25.6	100.0
K	27.5	25.0	23.3	24.2	100.0
L	26.2	21.3	24.6	27.9	100.0
M	26.9	22.1	25.0	26.0	100.0
Average	26.6	23.1	24.3	26.0	100.0

INSTRUCTION OF OBSERVERS

Five observers were used in the comparison. During the month prior to the test all five had been using the pantograph in regular

spring field work, having been uniformly trained and supervised in its use until the writer was convinced of their competence. If the observers were most proficient in any one, it was in this, the chart method. Most of them had little previous experience at listing and point-analyzing; but a full day (July 2) was given to preliminary trials and instructions in both methods, and it was felt that the techniques were mastered. In the course of these practice trials, standards of judgment in use of the list method were probably influenced by the results obtained in point-analyses. Inasmuch as almost any standard can be adopted for visual estimates, the later similarity in results by the two methods may be due to this fact.

It is manifestly impossible to say that these five observers constituted a representative sample of field assistants. The writer is confident that in ability they were at least not below average; they were all intelligent, well trained, and conscientious. Two were university graduates and three were students. To assume that the observed variability is due to incompetent personnel, then, is not so reasonable as to assume that some such variability may be expected in other, similarly high-grade assistants. It is noteworthy that one of the most experienced of the five men tended to make the most variable observations, and one of the least experienced the most consistent observations. The writer is led to suspect that variability in performance is closely associated with temperamental qualities.

An unavoidable limitation of the study was that the men knew they were being tested. Even though they were instructed not to let this consideration affect their work, and the writer believes they honestly tried to comply, it is scarcely to be expected that their results were wholly unaffected. A condition imposed in the trials was the rotation of recorders, so that in the first four trials no recorder accompanied an observer for more than one complete round of observations. That each man had to act both as recorder and observer was almost unavoidable. Only five men were available, and it was considered more advantageous to have five observers than to sacrifice two for full-time recorders, even though the use of five men did involve a greater risk of standardizing results. Care was taken to avoid making comparisons during the period of field work. Recorders were instructed not to compile any totals or to study the data or to comment about them in any way. It might be supposed that under some circumstances an observer's results would vary with different recorders, but such an effect is not discernible in the data from this study.

NUMBER AND NATURE OF TRIALS MADE

The purpose in having several trials on each quadrat by each man using each method was to obtain an estimate of the variability of individual observers. Circumstances which will be described prevented the attainment of this estimate of what biometricians call pure error.

In order to avoid effects of memory—which if operative would tend to reduce variability and the estimate of error—each quadrat was approached from a different side at each trial. While it is not possible to evaluate the success of this device, the observers were of the opinion that memory did not affect their work.

The original plan required each observer to make four trials of each method on each quadrat. Actually, six trials were made of the point method, the first four corresponding in time to the four trials by the chart and list methods. For the first two trials of the point method 400 points per square meter were projected. Fearing that this rate might not be sufficiently intensive to give the method a fair test, it was doubled, with the points still evenly spaced. Four trials were made at the 800-point rate, the first two corresponding in time to the last two trials by the chart and list methods. Records were kept in such a way that the pattern of points at the lower rate could be accurately segregated from these four trials. Data are therefore available for six independent trials at the lower rate as well as four at the higher rate. The additional trials complicate analysis somewhat, especially since the first two trials were at a lower rate than the last four.[6]

To have the work completed quickly was desirable, in order to minimize the change of vegetation with season. Trials were begun July 6 and completed July 23, 1936, covering an interval of 2½ weeks. It happens that the majority of observations in each trial fell inside a 4-day interval. There was no set sequence of methods, several trials of each being made on each day. Trial 1 corresponds roughly to July 6–9, trial 2 to July 10–13, trial 3 to July 14–17, and trial 4 to July 18–21. Trials 5 and 6 by the point method were made mainly July 22 and 23.

Ordinarily, no great change would have taken place in this period, but three unforeseen factors probably caused some fluctuation in density—drought, heat, and grasshoppers. The summer drought of 1936 was one of the most severe on record, and in order to have green grass on the quadrats it was necessary to water them. The countryside was suffering a grasshopper plague, and the small islands of green grass attracted grasshoppers by hundreds. Poisoning was of no avail, and finally it was necessary to place frames covered with screen or stretched burlap over the quadrats whenever they were not being worked. Intense heat, together with shade and moisture under the frames, probably resulted in forcing the grass; on the other hand, repeated handling of the vegetation, even though with great care, and a certain number of grasshoppers that somehow always managed to get under the frames, were more or less compensating factors. Judging from a series of photographs made July 9 and 21, there was not much change in the vegetation. On quadrat 1 vegetation may have been slightly denser and on quadrats 2 and 3 slightly sparser at the beginning of the trials than at the end.

This explanation is necessary in order to make clear the complexity of the factor trials. The variation between trials proved to be considerable, and it may be wondered whether it should be attributed to a progressive trend in observers' judgment or to alterations in vegetation. Apparently any great change in vegetation must be ruled out.

EXPERIMENTAL RESULTS

The source data, expressed in square centimeters of vegetation per square meter, are given in table 2. In order to study possible inter-

[6] In the tables of source data the results of each 800-point trial are split and expressed as two separate 400-point values. The 800-point value may be obtained by averaging each pair. Unless otherwise specified, trials 1 and 2 and the corresponding halves of trials 3 and 4, printed in roman type, are the point data used in the ensuing discussion.

actions between factors, analysis of variance (*13*) of all the data was attempted; but the individual components of the highest order interaction, used as error, proved to be so heterogeneous, apparently because of the great differences in quadrat density, that this scheme had to be abandoned. Even when separate analyses were made by quadrats, there was some likelihood of heterogeneity among the error terms, apparently because the chart method gave results so different from the others. Consequently, analysis is restricted to the methods separately for each quadrat, and the opportunity to study interactions statistically is lost. In the following discussion only those data are combined which appear to be reasonably homogeneous.

DIFFERENCES BETWEEN QUADRATS

A study of averages by methods on the three quadrats reveals that, although results may differ markedly with method, on the average the methods bring out the relative areas of grass in much the same way. The ratios between quadrats 1, 2, and 3 are close to 1.00 : 1.71 : 2.85 for all three methods.

TABLE 2.—*Individual estimates of short-grass area on quadrats 1, 2, and 3, in square centimeters per square meter*

QUADRAT 1

Method and trial No.	Estimate by observer—					
	B	D	E	G	L	Average
Pantograph-chart:	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
1	1,188	1,146	1,233	1,131	1,078	1,155
2	882	941	931	1,021	1,017	958
3	826	1,083	1,058	903	804	935
4	1,120	998	960	969	933	996
Average	1,004	1,042	1,046	1,006	958	1,011
Density-list:						
1	696	606	836	772	724	727
2	710	718	746	726	700	720
3	688	676	732	612	644	670
4	706	596	734	526	578	628
Average	700	649	762	659	662	686
Point-analysis: [1]						
1	950	750	850	800	550	780
2	600	700	400	450	650	560
3	1,250	750	900	750	550	840
	750	*750*	*600*	*750*	*800*	
4	700	450	500	600	600	570
	1,100	*700*	*600*	*600*	*550*	
5	550	550	550	550	550	----
	600	*700*	*650*	*600*	*900*	
6	700	550	500	550	450	----
	700	*650*	*500*	*750*	*600*	
Average [1]	875	662	662	650	588	688

See footnotes at end of table.

TABLE 2.—*Individual estimates of short-grass area on quadrats 1, 2, and 3, in square centimeters per square meter*—Continued

QUADRAT 2

Method and trial No.	Estimate by observer—					
	B	D	E	G	L	Average
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
Pantograph-chart:						
1	1,603	1,362	2,752	1,815	1,761	1,859
2	1,507	1,352	1,835	1,947	1,782	1,685
3	1,682	1,632	1,603	1,686	1,448	1,610
4	1,871	1,654	1,584	1,979	1,716	1,761
Average	1,666	1,500	1,944	1,857	1,677	1,729
Density-list:						
1	1,140	1,188	1,344	1,376	1,444	1,298
2	1,032	1,076	1,140	1,388	984	1,124
3	1,144	1,040	1,096	1,088	1,460	1,166
4	1,144	816	1,224	920	1,112	1,043
Average	1,115	1,030	1,201	1,193	1,250	1,158
Point-analysis: [1]						
1	1,300	1,100	900	900	800	1,000
2	1,300	1,100	1,400	1,500	1,000	1,260
3	1,100	1,900	1,300	900	1,600	1,360
	1,200	*1,300*	*1,200*	*1,200*	*1,100*	
4	1,400	600	1,300	1,500	1,000	1,160
	1,600	*900*	*1,100*	*1,200*	*1,200*	
5	1,200	600	1,200	1,100	1,300	----------
	1,400	*900*	*600*	*1,200*	*1,300*	
6	800	400	600	1,100	500	----------
	700	*800*	*1,000*	*600*	*1,200*	
Average [2]	1,275	1,175	1,225	1,200	1,100	1,195

QUADRAT 3

Pantograph-chart:						
1	3,259	3,275	4,596	3,957	2,298	3,477
2	2,464	2,092	3,798	3,088	2,936	2,876
3	2,385	2,960	2,994	2,712	2,672	2,745
4	3,105	2,890	3,082	3,697	2,063	2,967
Average	2,803	2,804	3,618	3,364	2,492	3,016
Density-list:						
1	2,280	1,656	2,760	2,556	2,384	2,327
2	1,616	1,648	1,820	1,608	2,036	1,746
3	1,940	1,284	1,968	1,312	2,064	1,714
4	1,632	1,272	1,868	1,496	1,996	1,653
Average	1,867	1,465	2,104	1,743	2,120	1,860
Point-analysis: [1]						
1	2,300	2,300	1,900	1,800	2,200	2,100
2	2,000	1,800	1,300	1,000	1,500	1,520
3	2,000	2,200	2,000	2,100	2,200	2,100
	2,400	*2,800*	*2,000*	*2,100*	*1,700*	
4	2,400	1,900	1,600	2,000	2,000	1,980
	2,700	*2,200*	*2,000*	*2,400*	*2,300*	
5	2,400	2,300	1,700	1,600	1,700	----------
	2,800	*1,900*	*2,200*	*2,300*	*2,200*	
6	1,900	1,400	1,200	1,900	1,800	----------
	2,400	*2,300*	*2,300*	*1,900*	*1,900*	
Average [2]	2,175	2,050	1,700	1,725	1,975	1,925

[1] The paired values represent separate components of the 800-points-per-square-meter rate. The upper figure, in roman type, corresponds to the 400-point rate in trials 1 and 2. Results of the 800-point rate may be obtained by averaging each pair.

[2] Average of values in roman type only, for comparison with other methods.

DIFFERENCES BETWEEN METHODS

Table 2 shows average chart values to be higher by about half than either list or point values. An examination of the individual data reveals that out of 60 cross comparisons, chart values are exceeded only four times, twice by list and twice by point values. One reason for similarity between the last two methods has already been suggested. In the data presented by Hanson and Love (*5*) in which results obtained by chart and list methods are compared, the values obtained by charting *Buchloë dactyloides* and most tufted grasses are, as here, higher. The reason is not far to seek. Few clumps of grass form a solid cover. Even when the greatest pains are taken, a pantograph can record only the larger openings, and so the numberless small ones, which aggregate a considerable area, are lumped in with the area of vegetation. But when used with care, the list and point methods do take these many small openings and marginal irregularities into account. This fact is not fully appreciated until one has worked with a point-analyzer; then he is astonished at the frequency with which a point may penetrate a dense tuft of grass without striking a single leaf.

It will be observed in table 2 that average point values are slightly higher than average list values. Is this difference significant? Table 3 analyzes the differences between individual values, and it is seen, from the probabilities given in the last line, based on *t*-tests (*3*), that observers B and D have a strong tendency toward higher point values. On the other hand, L apparently has a tendency, not quite so strong, toward lower point values. E and G show negative and positive tendencies, respectively, without marked consistency.

TABLE 3.—*Differences between individual point*[1] *and list values, in square centimeters per square meter*

Quadrat and trial No.	Differences for observer—				
	B	D	E	G	L
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
Quadrat 1:					
1	+254	+144	+14	+28	−174
2	−110	−18	−346	−276	−50
3	+312	+74	+18	+138	+31
4	+194	−21	−184	+74	−3
Quadrat 2:					
1	+160	−88	−444	−476	−644
2	+268	+24	+260	+112	+16
3	+6	+560	+154	−38	−110
4	+356	−66	−24	+430	−12
Quadrat 3:					
1	+20	+644	−860	−756	−184
2	+384	+152	−520	−608	−536
3	+260	+1,216	+32	+788	−114
4	+918	+778	−68	+704	+154
Average	+252	+283	−164	+10	−136
P value[2]	.006	.04	.10	>.9	.07

[1] The full estimate is considered in each case; i. e., trials 3 and 4 are at the rate of 800 points per square meter.

[2] A *P*-value of 0.5 indicates that the departure of the average from zero is just as likely to be the result of chance as of experimental causes, a "50–50" probability; a *P*-value of 0.05, that the departure of the average would be the result of pure chance in 5 trials out of 100. In general, the smaller the *P*-value the greater the probability of significant results.

The conclusion, then, is that the slight differences between means are not the result of differences between methods so much as differ-

ences between observers; another crew with more men of "L's" temperament would presumably have produced relatively lower point values. The practical significance of this conclusion is that observers may be expected to respond differently to different methods. Biometrically speaking, there tends to be an interaction between observer and method. The existence of such an interaction is a warning against attempting to convert individual records obtained by one method into terms of some other method.

As has been noted, the point method is subject to two sources of error—personal bias (from which, if practice coincide with theory, it should be free), and errors of sampling. How big a part does each play?

Since point-analysis records were so kept that the 400 points per square meter added in the last four trials can be segregated, each of these estimates can be separated into two parts. The variations between these parts provide an estimate of sampling error, as opposed to error derived from the observer-trial interaction. From separate analyses of variance, including a break-down of quadrat 1 into north and south halves, each 0.5 by 0.5 m., table 4 is derived. In general the paired variances are strikingly similar. The variation (12 degrees of freedom) designated "interaction" is neither greater nor less, consistently, than sampling variation (20 degrees of freedom), and one is led to conclude that the errors of personal bias by the point method are of the same order of magnitude as those inevitable in sampling.

TABLE 4.—*Sampling variances as compared with interaction variances of the point method, and probabilities of significant differences in magnitude*

Quadrat	Interaction variance	Sampling variance	*P*
Quadrat 1:			
Entire	16,219	21,750	.69
North half	63,542	44,500	.23
South half	33,792	40,500	.62
Quadrat 2	104,417	64,750	.17
Quadrat 3	52,750	123,750	.93

A practical problem that confronts the field worker with the point method is to know what number of point projections he should use in order to attain a desired degree of accuracy. If the points were distributed at random rather than in a rigid pattern, he would expect sampling accuracy to vary in inverse proportion to the square root of the sample size. Since in practice he is dealing with a rigid rather than a random pattern, in which each projection is not completely independent of every other, what may he expect? May he utilize sampling theory at all?

If sampling theory is applicable, we should expect the variation between 800-point values in the last four trials to be smaller than that between 400-point values by the inverse of the square root of 2, or 1.41. The last two columns in table 5, with four out of six values greater than 1.41 and two smaller, give no good reason for concluding the theory other than applicable in a broad rule-of-thumb way. Hence, within the limits of this empirical test, it would seem that after some preliminary determinations one might arrive at any

accuracy desired, by altering the frequency of point projection in accordance with theory.

TABLE 5.—*Effect of doubling point-analysis rate: Standard deviations from sampling by 800 and 400 points per square meter*

Quadrat No.	s_{800}	s_{400} (even)	s_{400} (odd)	$\frac{s_{400}\text{ (even)}}{s_{800}}$	$\frac{s_{400}\text{ (odd)}}{s_{800}}$
1	90.1	122.7	141.6	1.36	1.57
2	228.5	363.2	242.7	1.59	1.06
3	162.4	248.2	275.4	1.53	1.70

DIFFERENCES BETWEEN OBSERVERS

Some differences between the results of different observers are to be expected; yet, with every possible precaution taken, it is surprising how great such differences may be. For example, quadrat 3 as charted July 14 by B had 2,385 cm.^{2} of grass; but as charted on the same day by D it had 2,960 cm.^{2} or 575 cm.^{2} more. Three days later, B charted an area of 3,105 cm.^{2} on the same quadrat and D an area of 2,890 cm.^{2}, this time 215 cm.^{2} less! This example illustrates how observers tend to report results that are not only different but in many instances inconsistent.

At the same time, some rather fixed differences between observers are apparent, for, while there are many exceptions, E tended to be the highest and L the lowest man with the chart method, E the highest and D the lowest with the list method, and B the highest and L the lowest with the point method.

A summary of the significance of differences between observers, by quadrats and methods, is given in table 6, in which probabilities for the respective analyses of variance are computed with the help of Pearson's Tables of the Incomplete Beta-Function (*12*). Differences between observers are most clearly marked on quadrat 3, and least clearly marked on quadrat 2. A satisfactory explanation is lacking, except that quadrat 3, being the most difficult quadrat of the three, probably gave most opportunity for the exercise of personal judgment.

TABLE 6.—*Probabilities that differences between average areas reported by different observers and at different trials are due merely to chance*

OBSERVERS

Quadrat No.	Pantograph-chart method	Density-list method	Point-analysis method [1]		
			400(4)	800(4)	400(6)
1	.65	.07	.14	.07	.05
2	.28	.33	.96	.57	.66
3	.04	.006	.03	.01	.006

TRIALS

1	.009	.05	.02	.02	.002
2	.59	.11	.43	.02	.04
3	.15	.001	.003	.09	.003

[1] In the column headed 400(4), the third and fourth trials at 400 points per square meter are included with the first 2; the column headed 800(4) relates to the last 4 trials at 800 points per square meter; the column headed 400(6) includes all 6 trials at the rate of 400 points per square meter.

The data in table 7 will serve to illustrate some of the curious quirks of observers. They are selected because on two occasions each of the observers used all methods on the same quadrat the same day. Given these conditions, the same sort of variation could probably be demonstrated between any other observers. E's values by charting and listing are markedly lower on July 20 than on July 6 and his point values are almost the same. D's results, on other days than E's but similarly near the beginning and end of the work, are quite different; although he, like E, arrives at less area by listing on the later date, his areas by charting and point-analyzing are markedly higher.

DIFFERENCES BETWEEN TRIALS

On quadrats 1 and 3 results from the first trial average higher than results from later trials, and it is this difference which is reflected in the low P values for these quadrats in table 6. The likelihood of a major vegetation change, which has already been discussed, does not seem great enough to account for so sharp a difference. The writer is convinced, from taking part in the work as well as scrutinizing the results, that after the first two days, with the establishment of a work routine, there was a general swing among observers toward conservatism in estimate. This swing was by no means unanimous, but it was strong enough to produce much of the observed difference.

TABLE 7.—*Individual estimates of area of buffalograss on quadrat 3, in square centimeters per square meter, by two men, each having made all three estimates on the date given*

Observer and date	Pantograph-chart method	Density-list method	Point-analysis method
	Square centimeters	*Square centimeters*	*Square centimeters*
Observer E:			
July 6	4,596	2,760	1,900
20	3,082	1,868	1,800
Observer D:			
July 9	2,092	1,648	1,800
17	2,890	1,272	2,500

RELATIVE VALUE OF THE METHODS

Since in this comparison there is no absolute standard with which observed values may be compared, it is impossible to determine which method of the three comes nearest to giving the true values. The point method, because it is the most mechanical, might be expected to reflect vegetation area without bias, and perhaps it comes nearest of the three methods to doing so; but as the probabilities in table 6 indicate, there were consistent differences between observers using the point method, so that this expectation is not fully realized.

IN CONSISTENCY

Since we cannot know which method is most accurate absolutely, the next best thing is to know which is most consistent within itself. An appropriate measure of variability, the inverse of consistency, is the square root of the variance, or the estimated standard deviation

based upon the observed values. Estimates of standard deviations in the present test, since they are based on only 20 values (30 in the next to last column in table 8), are themselves subject to considerable error and can be considered only as rough approximations.

TABLE 8.—*Standard deviations, in square centimeters of vegetation per square meter, and as percents of the respective means*

Quadrat No.	Pantograph-chart method	Density-list method	Point-analysis method [1]		
			400(4)	400(6)	800(4)
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
1	90	54	151	125	90
2	292	152	345	207	228
3	491	216	210	234	162
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	8.9	7.9	21.9	19.5	13.6
2	16.9	13.1	28.9	28.2	21.2
3	16.3	11.6	10.9	12.4	7.8

[1] In the column headed 400 (4), the third and fourth trials at 400 points per square meter are included with the first 2; the column headed 800 (4) relates to the last 4 trials at 800 points per square meter; the column headed 400 (6) includes all 6 trials at the rate of 400 points per square meter.

In discussing averages it was pointed out that densities of the quadrats, although expressed on a different level by the chart from that by the others, are reflected similarly by all methods. A pattern is discernible in table 8 which shows variability to be more complex than this. The list method has, in the main, the smallest standard deviation; that is, of the three methods it tends to be the most consistent. Variability of both chart and list methods increases from quadrat 1 to 3, but variability of the point method is greatest for quadrat 2. The standard deviations expressed as percents of the means in the lower part of table 8 do not follow these same trends, which makes it seem unlikely that increases in variability of the chart and list methods from quadrat 1 to 3 are associated altogether with increasing density. Probably the low standard deviations of chart and list methods on quadrat 1 are associated with the predominance of small, compact tufts of grass on that quadrat, estimation of which tends to be standardized. Such definite tufts were lacking on quadrat 3.

Variability with increasing density increases somewhat more markedly by the chart than by the list method. This is probably associated with the decreasing definiteness of tuft outline from quadrat 1 to 3, making charting increasingly more difficult.

The tendency toward low standard deviations by the point method on quadrat 3 is probably associated with the fact that vegetation and bare spots were well distributed over the quadrat. The distribution on quadrat 1 was better than on quadrat 2 from the viewpoint of the point analyst, since the tufts, in general, were scattered, small, and open; whereas those on quadrat 2 tended to be larger and more compact. Thus, if a certain point hit a leaf on quadrat 2 there was more chance of its next neighbor striking a leaf also (differences in density aside) than there would have been on quadrat 1; and the same is true for a strike on bare ground. In other words, there was least likelihood of correlation between successive projections on quadrat 3

and most likelihood on quadrat 2; and the greater the correlation, the greater the likelihood of variability under the conditions of this test.[7]

IN RAPIDITY

Rapidity in a method is almost as important as a minimum of random error. The less time required for each quadrat, the greater the number of quadrats that can be examined, and the more reliable the resulting average will be. Moreover, the more rapidly the examinations can be made, the less the data will be influenced by changes in the vegetation with season. This is an especially important consideration where, as in the short-grass region, field work may be seriously curtailed by summer drought.

TABLE 9.—*Average field time per trial and average office time required for compilation* [1]

Method	Quadrat 1			Quadrat 2			Quadrat 3		
	Field	Office	Total	Field	Office	Total	Field	Office	Total
	Man-minutes	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*
Pantograph-chart	191	120	311	160	104	264	239	175	414
Density-list	62	5	67	45	5	50	51	5	56
Point-analysis:									
400 per meter [2]	58	3	61	37	3	40	48	3	51
800 per meter [2]	72	3	75	48	3	51	58	3	61

[1] Quadrat 1=0.5 m.²; quadrats 2 and 3=0.25 m.² each.

In the field a record was made of time consumed by each trial, and in the office, of the time required for its compilation. The averages, given in table 9, show that the chart method is 4 to 8 times as costly in total time as the other two.[8] In point of field time, a unit area of quadrat 3, which had about three times as much vegetation as quadrat 1, took about two and a half times as long to chart—that is to say,

[7] The explanations in this paragraph were tested with the aid of the field records. Inasmuch as this test may be of interest in application of the point-analysis method for studying the distribution of vegetation, it will be described.

Considering only the results of projections along the axis of the rack, occurrences of two hits together, two misses together, or a hit and a miss together were tallied. The frequencies of these occurrences were then compared with the frequencies which would have occurred if hits and misses had been distributed completely at random. Random distribution is given by weighing the expansion of $(p+q)^2$ by the proportion of the number of hits (p) and misses (q, where $q=1-p$) to the total number of trials. The term p^2S (where S is the total number) gives the number of expected hits, q^2S the number of expected misses, and $2pqS$ the number of expected hits and misses. The last four point-analyses (800 points per square meter) on each quadrat were used for the test. The averages of 20 deviations, observed minus expected, and their standard errors are as follows:

Quadrat	Miss and miss	Hit and hit	Miss and hit
1	-0.738 ± 0.454	*$+0.890\pm0.288$*	-0.153 ± 0.634
2	*$+.910\pm .388$*	*$+1.085\pm .370$*	*$-1.993\pm .695$*
3	$-.901\pm .574$	$-.174\pm .421$	$+1.074\pm .932$

The probability that these departures may be due solely to chance may be found by using a table of t such as given by Fisher (*3*). It will be found that P for the values in italics is less than 0.05, and these differences may therefore be thought of as being consistent enough to be real. For quadrat 1 there is a marked tendency for hits to fall together, but this tendency does not seem to affect the random occurrence of misses together, or of misses and hits together. This indicates numerous small, scattered tufts. For quadrat 2 there is a marked deficiency of misses and hits, and a tendency for hits to fall together and for misses to fall together. This indicates fairly large tufts and bare spaces; the deficiency probably arises from this cause and also from a tendency of the grass to be concentrated around the edges of this quadrat. (A single hit in the middle of the quadrat gives rise to two occurrences, a miss-hit and a hit-miss, but a hit by the point at the end of the rack can result in only half this much credit). Since the departures for quadrat 3 are well within the limits of expectancy, it is to be concluded that the distribution of grass on quadrat 3 is not proved other than random.

[8] Operation of the pantograph requires two men, but in the list and point methods only one need be used. It should be noted that the trials and time estimates used in this test are on a two-man basis, and hence allow for the list and point methods a considerably greater number of man-minutes than are needed in actual field practice.

working time is roughly proportionate to density—whereas by the other two methods the dense quadrat took only a little more than half again as long as the sparse one. Compilation of the list and point records took in every case less, usually much less, than one-twentieth the time required for chart compilation.

IN CONSISTENCY AND RAPIDITY COMBINED

In the preceding discussion it has been shown that the methods have differing degrees of variability and require different amounts of time, both of which are costs. Assuming the familiar relation to hold, that the squared standard error of the mean equals the squared standard deviation divided by the number of observations, we may determine the relative efficiencies (*3*), or inverse relative costs, of the three methods. Table 10 gives the efficiency components and their product in relation to the variability and time for the list method.

Strictly on the basis of time, the chart method is much less efficient than the others, and on the basis of variability, it is much less efficient than the list method. In its relation to the point method, the variability-efficiency of the chart method shows an interesting change from quadrat 1 to 3. On the scattered vegetation of quadrat 1, it is greater than that of the lower point-analysis rate and about equal to that of the higher rate; on the grouped vegetation of quadrat 2 it is lower than on quadrat 1, although still greater than the efficiency of the lower point-analysis rate; and on the dense vegetation of quadrat 3, it is far lower than the efficiency of the point method at either rate.

TABLE 10.—*Combination of the cost factors, variability (s^2), and time (T), relative to those of the list method; all terms expressed inversely as "efficiency"*

Method	Quadrat 1			Quadrat 2			Quadrat 3		
	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$
Pantograph chart	36.9	21.5	7.9	27.0	18.9	5.1	19.5	13.5	2.6
Density-list	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Point-analysis (400)	13.0	109.8	14.3	19.3	125.0	24.1	105.8	109.8	116.2
Point-analysis (800)	36.5	89.3	32.6	44.1	98.0	43.2	177.5	91.8	162.9

When the two factors are combined, the chart method is seen to have between one-twelfth and one-fortieth the efficiency of the list method, its relative efficiency becoming less as the vegetation increases in density and complexity. Depending upon sampling intensity, the relative efficiency of the point method varies here from one-seventh to more than half again as much as that of the list method, and, in contrast to the relative efficiency of the chart method, increases with increasing vegetation density. The efficiency of the point method can be considerably increased on vegetation of low density by increasing the number of points; that is, the decrease in variability from doubling the number of points more than makes up for the increase in time cost. On vegetation of higher density this increase in efficiency is progressively less marked.

ADAPTABILITY TO VARIED USE

The merits of these methods should be examined also in the light of the principal uses to be made of them. If comparison is to be made of the effects of known grazing treatments upon short-grass range over a period of years, including a record of the amount and kind of vegetation resulting under each treatment, and if data are desired on such ecological problems as conditions which govern seedling establishment or competition between individual plants of different species, periodical mapping may be necessary, and the chart method is the appropriate choice. If, on the other hand, the principal object is to know accurately magnitude of changes, rather than detail of processes involved in them, a more rapid, more accurate means, such as the list method, is desirable.

The chart method, besides providing a kind of pictorial history, showing the location and shape of each plant in relation to all the others, permits correction of many misidentifications. Chart data, however, are not always expressed in consistent terms, so that if one wishes to speak of spot symbols in terms of area, he must use an arbitrary conversion factor.

The list method, while it proved most efficient in this study, may not prove so in general application unless its use is carefully controlled. Estimates without a sound standard of reference are likely to vary between wide, and even wild, extremes. Hence it is necessary, in applying the list method, to provide some means of standardization. In the present test standardization was achieved by all observers working together in preliminary trials, and their concept of plant density was probably strongly influenced by the use of the point method, which they considered to be a more objective method than the others.

The principle of the point method was tested during the winter prior to the present test on quadrat charts on which the areas of outlined vegetation had been determined. About 400 intersections of regularly spaced lines were adopted as points, and it was found that the percentage of points falling within outlines of the principal species agreed closely with the area percent of those species. Under necessity to prepare a quick summary of uncompiled chart areas later, the writer applied the same method and rapidly made compilations sufficiently accurate for the immediate purpose. Abell (*1*) has recently described an application of the same principle to determine the areas of irregular figures on maps.

Another possible use of the point method, not touched upon in this paper, is the direct determination of volume by the total number of hits, that is, hits at all distances above the ground (*7*, *8*).

The variability of any method is probably at a minimum on an unobstructed quadrat such as the ones used in the present test. On many short-grass ranges, inclusion of cactus and sagebrush is unavoidable if the quadrats are intended to be truly representative. These plants interfere with operation of the pantograph and often require that the density-list frame be held at some distance from the ground. Tests made in the summer of 1937 showed that when the density-list frame is raised 7 inches off the ground, the variability of repeated estimates is markedly increased. Provided that sufficiently

long sliding pins are obtainable, the point method is probably superior to the others under such conditions.

The three methods are capable of improvement in varying degree. A more rigidly constructed pantograph would reduce some though not the most important errors of the chart method. Doubtless, greatest improvements are possible in the point method, by using better sighting devices to minimize the number of doubtful hits, by using longer pins which slide more smoothly, with finer harder points, and by reducing the awkwardness and weight of the apparatus. The apparatus as used in this comparison was bulkier than that used by New Zealand workers, but the sharpened uprights of the instrument used by Levy and Madden (*8*), when repeatedly driven into the soil in an arid climate, probably would affect the quadrat environment seriously. The even simpler device diagramed by Fenton (*2*) has a similar objection and seems less adapted to precise work.

SUMMARY AND CONCLUSIONS

Three methods of quadratting short-grass vegetation—the pantograph-chart, density-list, and point-analysis methods (referred to as "chart," "list," and "point")—were tested on three typical short-grass quadrats, of low, intermediate, and high density. Five trained observers used each method four times on each quadrat, except that the point method was used six times at two different intensities. A 2-week period was required for the comparison, during which, it was concluded, no appreciable changes in the vegetation took place.

On an average, the methods reflected the marked differences in grass area between quadrats similarly, although with differing absolute values.

With all methods, consistent differences between observers were most evident on the quadrat with grass of highest density and most matted habit.

One observer, it was found, may record consistently greater areas by one method than by another, and another observer may record lesser areas by the first method and greater areas by the second; thus there may exist an interaction between observer and method.

In contrast to consistent differences between the results of different observers, inconsistencies, sometimes of considerable magnitude, appeared within the work of a given observer, in spite of elaborate precautions in training and standardization.

Areas by the chart method tended to be 50 percent greater than areas by the other methods, and the chart method proved to be less consistent than the list method on all quadrats and more consistent only than the lower-rate point method on the low- and medium-density quadrats. Since it required much more time than the list and point methods, its net efficiency, within the limits tested, varied from one-half to less than one-fiftieth of theirs.

Areas by the list method tended to be similar in magnitude to those by the point method, which may be due to preliminary standardization practice. The list method gave more consistent results than the others (except for the point method on the high-density quadrat). It required about the same amount of time as the point method. Its net efficiency was much higher than that of either of the other methods

on the low- and intermediate-density quadrats, but lower than that of the point method on the high-density quadrat.

The two components of variation in the point method, sampling error and personal bias, were of about the same order of magnitude in this test. Although the points are not projected at random, one may arrive fairly close to any desired accuracy within the limits tested by altering the number of projections in accordance with sampling theory. As a corollary, since the method is a rapid one, its efficiency can be increased by increasing the number of projections. The benefit of such an increase was most marked with vegetation of low density.

In view of the results of this comparison, it is suggested that for estimating area of short-grass vegetation on intensively studied, permanent quadrats which are intended to sample the effects of grazing treatments, the density-list method, carefully standardized, be applied. The point-analysis method, pending its further development, may well be used for training and standardizing observers in the density-list method. The pantograph-chart method should be reserved for those studies in which the greatest need is a detailed graphic record of the vegetation.

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